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The Memorandum of Understanding between the Ministry of Health of Belize and USUHS was signed in December 1989. Two USUHS MTM&H students conducted research and obtained clinical experience in country. In August 1990, Jorge Palanco, MD received the MPH from USUHS. The renovation of the CML was completed in October 1990. The dedication of the CML and the ERC took place in November 1990. The preliminary analysis of the sera obtained in the febrile illness study indicate a significant prevalence of antibodies to many arboviruses. An outbreak of leishmaniasis in the BDF was investigated. The information obtained was incorporated in preventive measures, resulting in a significant decrease in cases. During the study of the ecology of mosquito fauna, a total of 2600 individually reared specimens (with larval and/or pupal skins) were obtained from 226 individual collections. Specimens from approximately 90 collections have been identified. Preliminary analysis of plants associated with the aquatic environments demonstrate maior clusters of habitat-types

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FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR56.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

INTRODUCTION

The objective of this grant is to establish, operate and manage research and teaching programs in overseas locations where the USUHS has established bilateral research agreements. These centers are to serve as sites wherein research projects of programmatic interest to the USAMRDC in the field of tropical infectious diseases can be conducted by USAMRDC and USUHS personnel in collaboration with host national counterparts. The program also provides the opportunity to transfer technology to the host-country scientists and technicians through short-term and degree-granting programs. It also provides USUHS medical students, Master of Tropical Medicine and Hygiene students and Doctoral Candidates in Medical Parasitology and Vector Biology opportunities to obtain practical experience with tropical infectious diseases of the Western Hemisphere.

In Belize, the primary objective is to establish and maintain an Epidemiological Research Center (ERC) for infectious disease research and teaching in the Ministry of Health, Central Medical Laboratory (CML), Belize City.

Original research objectives:

a) Determine the etiology of acute febrile illnesses and jaundice; determine antibody prevalence against various arthropod-borne viruses (e.g., EEE, VEE, WEE, SLE, MAY, dengue, YF, VSV, etc.), leptospirosis, hepatitis A, B, C, D, by age, sex, ethnic group, and geographical location; maintain surveillance for epidemic disease due to arthropod viruses, especially dengue.

b) Determine prevalence of HIV and HTLV-1 infections in selected populations.

c) Determine patterns of drug resistance of *Neisseria gonorrhea* in various regions.

d) Determine patterns of malaria transmission; maintain surveillance for chloroquine-resistant *Plasmodium falciparum.*

e) Determine vectorial capacity of putative malaria vectors.

f) Address epidemiologic targets of opportunity [e.g., leishmaniasis in the Belize Defense Forces (BDF); causes of febrile illnesses in British Forces Belize (BFB), U.S. Army Corps of Engineers, etc.].

g) Determine the effectiveness of repellents and fabric impregnants for protection of deployed troops from endemic vector-borne diseases

h) Validate the remote sensing models developed for use in predicting temporal and spatial changes in malaria vector abundance in Mexico, in a second ecologically similar area (i.e., Belize).

Background

Belize is located on the east coast of Central America at the base of the Yucatan Peninsula, bordered on the south and west by Guatemala, on the north by Mexico, and on the east by the Caribbean Sea. The jungle-covered Maya Mountains occupy the southwestern part of the country, rising to 1122 ft. at Victoria Peak; the rest of the country is low, crop or scrub-covered coastal plains. Belize was founded as a buccaneer settlement and entreport. Belize is an English-speaking country, which gained independence from Great Britain in 1981. The population of approximately 195,000 people is a mixture of Mayans, Garifuna (Afro-Amerindian), Blacks, East Indians, Creole and Caucasians. About half of the population lives in the largest city, Belize City, on the Caribbean coast. The capital, Belmopan (population about 5,000) was built inland as a Federal District after a devastating hurricane in 1961 destroyed the former capital, Belize City. Several smaller cities; i.e., Punta Gorda, Dangriga, Hill Bank, Orange Walk, San Ignacio and Indian Church, are scattered through the coastal plain.

Medical care is provided by a socialized medical system, centered around local health clinics and district hospitals in the smaller cities and towns and a large central hospital in Belize City. Emergency cases (mainly surgical) are brought to Belize City Hospital for care by air ambulance, provided to the government by a Christian groups that has established missions throughout the country. The Belize City Hospital was built about 1930 and is a two-story building backed on the sea. It has about 200 beds, of which about 60% are dedicated to acute surgical patients. The hospital is divided into male, female and pediatric wards for both surgery and medicine. There are also neonatal and intensive care wards. A small chemistry, hematology and immunohematology laboratory for acute diagnostic procedures is located in the hospital. The majority of diagnostic and public health laboratory procedures are performed at the Ministry of Health, Central Medical Laboratory (CML) located about 3 miles north of the city. Current laboratory capabilities include: malaria smears (about 29,000/year), bacteriologic cultures, routine biochemistries, and HIV antibody testing using commercially available ELISA kits.

Febrile Illnesses

Little is known about infectious diseases specific to Belize. Based on limited information from Belize and other Central American countries, it may be inferred that such diseases are common in Belize. Yellow fever has been reported from the Yucatan[1], dengue and malaria are endemic in Belize[2], and cutaneous leishmaniasis, in almost epidemic numbers, has been reported in British troops stationed in Belize[3]. Cutaneous and visceral leishmaniasis have been reported in Honduras and Guatemala[4]. Leptospirosis, and Venezuelan

equine and St. Louis Encephalitis have also been found in neighboring countries[5]. Enterically transmitted non-A, non-B hepatitis has recently been identified in Mexico. The Statistics Department at the hospital reported yearly admissions over the past several years for enteric fever of 4 to 6 cases; jaundice, 60 cases; and fever of unknown cause, about 150 cases. A great deal needs to be done to determine the prevalence and incidence of tropical infectious diseases agents in Belize.

Cases of unexplained fever were selected by trained Belizean collaborators from patients over12 years of age presenting at the Belize City, San Ignacio and Orange Walk Hospitals. Patients with sickle cell disease, meningitis, dysentery, or evidence of peritonitis, wound infection, pneumonia, tuberculosis or HIV infection were not included in the study. Thick and thin malaria films were prepared from finger sticks and examined. Patients positive for malaria were listed but not studied. Patients selected for the study were divided into two groups, those with and those without jaundice. A systematic clinical exam was performed and blood was obtained for diagnostic tests. Sera were analyzed for the presence of antibodies to arthropod-borne viruses, leptospirosis and hepatitis.

Leishmaniasis

Cutaneous leishmaniasis is a zoonotic disease transmitted to man by human-biting female phlebotomine sand flies. Many *Leishmania* strains belonging to at least four species are capable of causing human disease. The clinical manifestations of the infection are primarily species-dependent, but a number of poorly defined host factors may influence disease expression. Flagellated promastigote-stage leishmanial parasites develop in the gut of female sand flies and are transmitted to a vertebrate host during a blood meal. Promastigotes rapidly parasitize macrophages, change to intracellular amastigotes, and multiply. In the absence of specific immunity, they circulate to regional or systemic reticuloendothelial system and cause sores and other manifestations after an incubation period of 1-6 weeks or longer.

In the New World particularly, cutaneous leishmaniasis presents with skin lesions as a major manifestation, but regional lymphatic chains are frequently involved as well . Typically, erythematous macules appear at the inoculation sites up to months after infection, followed by papules. The papules subsequently become nodular and may ulcerate to form well circumscribed ulcers with indurated margins and necrotic eschar-covered bases. In the absence of bacterial superinfection, which is unusual, the lesions are not painful. Satellite lesions may be found a small distance from the primary ulcers in otherwise normal-appearing skin. Some strains appear to cause a milder form of the disease, and the primary lesions may not ulcerate but instead present as nodules, papules or eczematous plaques. Non-tender nodules and inflammation can develop along draining lymphatics. Regional lymphadenopathy is common, and parasites can be isolated from these nodes.

Metastatic spread in the New World is generally associated with *L. braziliensis* and may occur months or years after acquisition in up to 80% of infections. Infections caused by *L. mexicana*, *L. peruviana*, and some strains of *L. braziliensis* are thought to cause localized disease without mucous membrane involvement. Spontaneous healing appears to be quite common with *L. tropica*, *L. major* and *L. mexicana*, except when the ear (pinna) is involved.

Leishmania mexicana is primarily distributed in Mexico, Belize and Guatemala. It is commonly (in 40% of cases) associated with lesions limited to the pinna of the ear and classically occurs in those harvesting gum from chicle plants; hence the name, "chiclero's ulcer." Forest rodents are the natural reservoir for *L. mexicana*. *Leishmania braziliensis* is thought to occur in Guatemala, though its presence is not as well documented as that of *L. mexicana*. In the Old World, cutaneous leishmaniasis is typically ulcerative and often heals spontaneously within several months. Spontaneous healing of New World lesions is much less predictable. Certain South and Central American species, notably *L. braziliensis*, may result in a slowly healing primary ulcer and late development of a mutilating infection of the upper respiratory tract (mucocutaneous leishmaniasis or espundia). A rare form known as diffuse cutaneous leishmaniasis is characterized by massively parasitized non-ulcerated nodules, specific cutaneous anergy, and a poor response to therapy. It occurs in both the New and Old Worlds. Serious illness with leishmaniasis has been reported as a manifestation of HIV infection.

The differential diagnosis of cutaneous leishmaniasis includes pyogenic bacterial, mycobacterial (*Mycobacterium marinum*), fungal (blastomycosis, sporotrichosis, histoplasmosis) and spirochetal (yaws and syphilis) infections, plus lupus erythematosus, sarcoid and malignancy.

Following the outbreak in the Belizean Defence Force in 1990, cases have been closely monitored.

Gastroenteritis

Rotavirus is a major cause of severe diarrhea in children. Worldwide it is estimated that there are 140 million cases and 1 million deaths[6]. Approximately 50% of infants and young children hospitalized with diarrhea are infected with rotavirus[7]. Transmission is thought to be by the fecal-oral route. Seasonality is a poorly understood feature of rotaviral enteritis. In temperate climates, rotavirus is detected most frequently in the winter. In the tropics, however, it is detectable all year round with seasonal trends that are less distinct[8]. LeBaron, et al.[6] and Glass[9] reported the rotavirus peaks (2 highest consecutive months) for Mexico, Belize and Guatemala would occur in the October-November time frame.

Hepatitis

In May 1991, concern that cholera may be spreading north through Central America prompted officials of the Belizean MOH to initiate an active search for possible cases. In the Cowpen area of southeast Belize there were no reports of illness resembling cholera, but banana farm workers and local health officials were concerned about the large number of people who were or had been ill with hepatitis. In some cases people had diagnosed themselves as having hepatitis, based upon the occurrence of jaundice. For others, the diagnosis had been made by local health professionals.

Cowpen residents considered the appearance of hepatitis to be an annual occurrence, associated with the dry season, and attributed the disease to the poor quality of water available. However, many believed the 1991 dry season was more severe and longer than usual and the numbers of cases, particularly in young adults, was greater than they had seen before. Concern about hepatitis increased when the death of a young, pregnant woman and her child were attributed to the disease.

The Belizean MOH responded to the apparent increase in hepatitis cases by requesting assistance from the Belize - United States Epidemiologic Research Center (ERC) in Belize City. In collaboration with the Uniformed Services University of the Health Sciences (USUHS), and with laboratory support from the Walter Reed Army Institute of Research (WRAIR), a preliminary investigation was done in May 1991 to try to establish a diagnosis. The May investigation by the Belizean team established the presence of hepatitis in the Cowpen area and the need for further study. This was followed in June 1991 by an expanded effort to identify and define cases and to do a cross-sectional study of hepatitis markers and related variables in banana farm workers and their families.

Mosquito Ecology

The numbers of malaria cases in Belize, as in most Central American countries, have increased dramatically in recent years. Belize presently reports the highest annual parasite index of any Central American country[10].

The vectorial roles of anopheline species found in Belize are poorly defined, even though several endemic species are known vectors in other geographic areas. *Anopheles albimanus* is currently considered the primary malaria vector in Belize. Although there is evidence that this species is indeed a primary vector, the roles of other species occurring on the coastal plain, or in the foothills, or mountainous areas should be determined. Improved understandings of roles and biologies of the different vectors in different geographical areas will undoubtedly lead to improved targeting of malaria control interventions.

Mosquito surveys and characterization of their aquatic habitats were

conducted in Belize during wet and dry seasons of 1990 through 1993. The primary objective was to collect taxonomic series of mosquito species found in Belize, with secondary emphasis on quantifying the presence/absence, abundance, and phytoecological relationships of anophelines in various aquatic habitats.

Tick-borne fever

Analysis of sera from the 47 workmen at the Caracol archeological site has not been completed by the USAMRIID lab. Preliminary results showed 7 of the 47 positive for Lyme disease. However, two of these specimens are also positive for syphilis, a known cross reactor. Antibodies to other infectious diseases will cross react in this enzyme linked immunosorbent assay (ELISA); therefore, positive results with Borrelia burgdorferi need to be confirmed with other diagnostic tests.

Cholera

The first case of cholera in Belize was confirmed on 10 JAN 92, from Punta Gorda. By 28 JAN 92, two more cases were confirmed from Punta Gorda. COL Krieg accompanied an epidemiological team investigating these cases. The team included epidemiologists from Guatemala, Jamaica, and Belize. All three of these first cases seemed to have been imported, probably from Guatemala.

More cases of cholera continued to be reported from Toledo District and, later, from Cayo District. By 7 SEP 92, there had been a total of 29 cases (including two deaths) reported. As of 26 NOV 92, there had been a total of 189 cases (including three deaths) reported from Belize. These cases were mainly from southwestern Toledo District, but a few cases had been reported from several towns in Cayo District (mainly along the Western Highway) as far east as Belmopan.

At the request of the Permanent Secretary for Health Affairs, Mr. Fred Smith, the ERC developed a detailed plan and a "costed" list of supplies and equipment needed to train District Med. Techs. in proper identification of cholera organisms in medical samples. These were offered to the CML on 13 October, along with the services of two ERC technologists to do this training. A limited amount of such training has since been started by CML personnel.

Chagas

American trypanosomiasis is a zoonotic disese caused by the protozoan *Trypanosoma cruzi* , which is transmitted to animals and humans via the triatomide (kissing) bug. Though this is still the most common mode of transmission of Chagas' in high-risk rural areas of endemic countries, studies have now shown that another mode of transmission is by the transfusion of contaminated blood (17). Though many infected people have no clinical manifestations, the acute stage generally occurs in children, while the chronic

stage appears later in life. The acute stage is characterized by fever and enlargement of the liver and spleen. During the chronic stage, life-threatening arrhythmias, cardiomyopathy, megacolon or megaesophagus may develop(18). It is estimated that there are 16 to 18 million people that are infected throughout Mexico, Central and South America (17). Most of these people live in poor rural or suburban areas where economic hardships and inadequate living conditions force them to migrate to other neighboring countries, including Belize.

Previous studies have shown that infected migrants have had an effect on non-endemic countries like the U.S., where it estimated that 100,000 individuals are suspected of carrying the disease (17,19). In one study in Central Americans in the Washington, DC area, 4.5% were found to have antibody to *T. cruzi*, and half were confirmed by xenodiagnosis (20). Concerns about transfusion-associated Chagas' disease in the U.S. are increasing (5,6).

Limited studies in Belize have confirmed the presence of *T. cruzi* in infected animals and infected triatomide bugs (20). However, studies of the human population have not been done. Clinicians suspect the presence of the disease based on clinical presentations of dilated cardiomyopathies. However, no pathologic or serologic diagnosis capabilities are available.

Laboratory diagnostic tests for Chagas' disease include xenodiagnosis, complement fixation, indirect immunoflorescence, hemaglutination inhibition, and direct agglutination tests, and more recently ELISA tests (17). ELISA testing is a convenient, sensitive and specific method for detecting antibodies to Chagas' disease. Testing with an early ELISA test indicated a sensitivity of 81% in acute disease and 100% in chronic disease, similar to latex agglutination and indirect immune florescence tests. However, there may be some cross reactivity with leishmanial antibody (23). An ELISA developed by the Abbott Corporation has been found to have a sensitivity of 100% and a specificity of 99.0% (24).

More recently, a second generation test has been developed by Abbott. This test is said to have a sensitivity of >99.99% and a specificity of >99.98%. Using this test, 13 (0.166%) of 7,835 persons in the U.S. with hispanic surnames were found to have antibodies to *T. cruzi* (25).
The test compares favorably with the IFA and indirect hemaglutination test in sera from Central America (26).

RESULTS AND PROGRESS

Administrative

Management changes, effective 11 March 1993, were made when Larry W. Laughlin MD, PhD, assumed responsiblilty as Co-Director, Epidemiologic Research Center, Belize. This change was made because Col. Richard Kreig retired in June, 1993 and no onsite replacement was available. While active recruiting for this position is ongoing, it was thought advisable to appoint an off-site Co-Director. In addition, an on-site Laboratory Supervisor, Ms Linda Reyes, was appointed, in order to provide day to day leadership at the ERC. Mr. Fred Duncan remained an invaluable USUHS administrative assistant for this program. Dr. Vanzie has continued as the Belizean Co-Director after the recent Belizean elections and the appointment of a new Minister of Health.

U.S. Embassay relationship changes have also taken place since Dr. Laughlin's visit in January 1994. National Security Directive (NSD) 38 requires all U.S. Embassys to downsize. Several State Department programs or programs receiving administrative support have been recently downsized or deleted. The Ambassador, while lauding the ERC program and promising continued diplomatic support, informed Dr. Laughlin that the on-site position was to be deleted. Furthermore, because of reductions in his staff, administrative support for the USUHS-funded ERC quarters and ERC vehicles would have to be terminated in the near future. This will directly impact the TAD costs for U.S. project travel of U.S. personnel, who can no longer take advantage of the USUHS quarters.

After short courses in accounting and financial management, Mrs. Rosie Miller was advanced to Administrative Stenographic Secretary.

MS-IV Laura Bulmahn completed an elective rotation in tropical medicine at the ERC, while also providing support for the febrile illness protocol. MTM&H student, Dr. Alicia Scott-Wright, completed her OCONUS rotation, leading a study of the hepatitis-B serologic status of women attending prenatal clinics in different regions of Belize.

In response to a request by the Belizean Ministry of Health, Department of Preventive Medicine and Biometrics,USUHS provided a 3-day workshop entitled "Research Methodology". Workshop

participants included Drs. Legters, Laughlin, Dixon, Bryan from USUHS and each of our Belizean MTM&H graduates, Drs Kishore, Pitts, Polanco and Craig. Dr. Vanzie, MD, PhD graduate of Tulane School of Public Health, also presented. The workshop was well attended by MOH physicians who had interest in or would likely have assignments where research methodology would be useful. The Belizean MOH continues to support and encourage research at all levels of physician development.

Ms. Shilpa Hakre attended the annual meeting of the American Society of Tropical Medicine and Hygiene in Atlanta, Georgia, and participated in a pre-meeting course entitled "Tropical Medicine Update: A Bench to Bedside Perspective on New and Re-Emerging Tropical Infectious Diseases". Ms. Linda Reyes and Dr. Polanco attended a "Malaria Control with Remote Sensing Technology" planning meeting at USUHS, with the aim of submitting a grant proposal for NASA funding. ERC staff made poster presentations at the National Agriculture Show, Belmopan, Belize, and at Health Week, Belize City. Posters focused on the ERC work in diarrheal disease and the preventive measures that could be taken by the lay population. ERC staff members attended local workshops held by the National Prevention and Control Committee for the Cholera Epidemic and on Quality Control, Safety and HIV Testing given by the Caribbean Epidemiology Centre (CAREC). Dr. Joe Bryan made four trips to the ERC to coordinate the current research programs. Dr. Donald Roberts and his staff and collaborators made three trips to lead the entomological research efforts. Drs. Legters and Laughlin each made individual trips for administrative purposes.

Current staffing with percentage of effort on active protocols:

Llewellyn J. Legters, M.D.,MPH - 10%
J. Fred Duncan - 60%
CAPT Larry W. Laughlin, MC, USN - 15%
CDR Joe P. Bryan, MC, USN - 40%
Donald R. Roberts, Ph.D. - 15%
Ruth Jaramillo, B.S., Mt (ASCP) - 100%
Linda G. Reyes, B.S. - 100%
Shilpa Hakre, B.S. - 100%
Rosita Burke - 100%
Ernest Black - 100%

Scientific Programs and Publications

Belize, ERC

1. Hoffman KJ, Gaydos JC, Krieg RE, Duncan JF, Macarthy PO, Ticehurst JR, Jaramillo R, Reyes LG, Sjogren MH, Legters LJ. Initial report of a hepatitis investigation in rural Belize. Transactions of the Royal Society of Tropical Medicine and Hygiene 87:259-262, 1992. (Enclosure 1).

A paper was also presented at Prevention 93 in Atlanta, GA, April 17-20, 1993 and at the 37th Annual Meeting of the Commonwialth Caribbean Research Council Meeting in Curacao, 22-25 April, 1992.

This study identified high rates of endemic hepatitis in a population of banana plantation workers. While high rates of HAV and HBV seropositivity were identified, the etiology of a significant percentage of acute cases could not be determined. These results suggest that a new type of hepatitis may be circulating in this population. A follow-on study to isolate and characterize a new hepatitis virus(es) will be a major focus of next year's scientific program.

2. Craig PG, Bryan JP, Miller RE, Reyes L, Hakre S, Jaramillo R, Krieg RE. The prevalence of hepatitis A, B and C among different ethnic groups in Belize. Am J Trop Med Hyg 49:430-434, 1993. (Enclosure 2). A paper was also presented at the 38th Annual Commonwealth Caribbean Medical Research Council Meeting, Apr 21-24, 1993, Trinidad. This resulted from MTM&H research project of Dr. Peter Craig.

This study identified very high rates of HBV seropositivity and antigenemia in one ethnic group of the BDF. To confirm this observation and explore possible explanations, this year's MTM&H student completed the next listed study.

3. Scott-Wright A, Hakre S, Bryan JP, Jaramillo R, Reyes L, Cruess D, Macarthy P, Gaydos J. Seroprevalence of hepatitis B, syphilis and HIV-1 in women attending prenatal clinics in Belize, an independent MTM&H project for Alicia Scott-Wright, MD, conducted during the summer of 1993. It has been submitted for consideration for presentation at the Commonwealth Caribbean Research Council Meeting for 1994 and at Prevention 94 in Atlanta, GA (enclosure 3) and is ready to be submitted to a journal.

Dr. Scott-Wright's work confirmed relatively high rates of HB core antibody in the Garifuna ethnic group, but found only one women positive for HBsAg. Risk factors for HB infection were number of people in the home, number of sex partners, and age of first intercourse.

4. **Hakre S, Reyes L, Bryan JP. Seroprevalence of hepatitis markers in health-care workers in Belize. Abstract being prepared for submission.**

This study further defined the epidemiology of hepatitis infections in Belize. These data have provided an important service to the Belizean MOH as hepatitis vaccination policies are now being formulated for health care workers on the basis of this study.

5. Bryan JP, Craig PG, Macarthy P, Reyes L, Hakre S, Jaramillo R, Legters L. Randomized comparison of 5 and 10-ug doses of two recombinant hepatitis B vaccines. Abstract 665. Presented at the 33rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy New Orleans, October 17-20, 1993 (Enclosures 4 & 5).

These data have contributed to cost-effective HB immunization practices, a subject of interest to both in Belize and the United States.

6. Jaramillo R, Gabourel I, and Bryan JP. Testing for Chagas Disease in 3 populations in Belize.

Presented at the Belize Medical and Dental Association Meeting, September 1993 and submitted for presentation to the American Society for Microbiology meeting in Las Vegas, April 1994 (Enclosure 6).

Sera from the BDF, persons living in the Cowpen area, and blood bank specimens have been tested for antibody to T. cruzi, the agent of Chagas' disease. As transmigration populations from *T. cruzi* -endemic areas continues throughout the Western hemisphere, transfusion trypanosomiasis will become an increasingly important issue. This study will contribute to the search for reliable diagnostic blood bank assays and procedures.

7. Roberts DR, Chan O, Pecor J, Rejmankova E, Manguin S, Polanco J and LJ Legters. 1993 Preliminary observations on the changing roles of malaria vectors in southern Belize. J Am Mosq Control Assoc. (In press). (Enclosure 7).

Abstract and presentation at the Annual Meeting of the American Mosquito Control Association, Sheraton Harbor Place Hotel, Fort Myers, Florida. April 18-22, 1993.

8. Harbach RE, Roberts DR, and Manguin S. Variations in the hindtarsal markings of Anopheles darlingi (Diptera: Culicidae) in Belize. Mosquito Systematics, (In press) (Enclosure 8).

9. Roberts DR, Paris JF, Manguin S, Harbach RE, Woodruff R, Rejmankova E, Polanco J, Wullschleger B, and LJ Legters. Use of satellite multispectral image data for locating malaria vectors in Belize. Submitted to Science.

10. Rejmankova E, Roberts DR, Harbach RE, Pecor J, Peyton EL, Manguin S, Krieg R, Polanco J and L Legters. Environmental and regional determinants of Anopheles (Diptera: Culicidae) larval distribution in Belize, Central America. Environmental Entomology 22(5):978-992, 1993.

11. Manguin S, Peyton EL and Loayza RF. 1993. Population genetics of Anopheles pseudopunctipennis, vector of malaria in Central and South America. American Mosquito Control Association, (18-22.IV.93), Fort Myers, Florida.

12. Roberts DR, Manguin S, Rodriguez MH, Rejmankova E, Spanner M, Beck L. Remote sensing technology and malaria control. Invited Presenter in the Symposium, "Future Predictions and Need," of the First International Congress of Vector Ecology held in San Diego, CA 3-8 October 1993.

13. Roberts DR, Paris JF, Manguin S, Harbach RE, Woodruff R, Rejmankova E, Polanco J and Legters L. The use of remote sensing and landscape features to accurately predict the presence and abundance of two malaria vectors in areas of Belize. Presented at the joint annual meetings of the Am Soc Trop Med & Hyg and Am Soc Parasitol held at Atlanta, GA., October 1993.

Dr. Roberts and colleagues continue to be extremely productive in both classical and avant garde malaria related entomology studies. Recent significant observations are: a) documentation of the *A. darlingi* in Belize, an important malaria vector, and b) cutting edge remote sensing technology which provided landscape epidemiology that was highly predictive of malaria vector activity. This remote sensing technology may have an enormous cost-effective impact on malaria control programs in the third world and the U.S. Department of Defense.

Pakistan, PULSE

1. Bryan JP, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, Duncan J, Rafiqui AR, Malik IA, Purcell RH, and Legters LJ. The seroepidemiology of epidemic hepatitis E in Pakistan as measured by a new and sensitive ELISA. Submitted to JID (Enclosure 10).

This manuscript and the following three presentations attest to the value of materials collected during outbreak investigations. The Sarghoda, Pakistan hepatitis epidemic has provided the data and materials for the development of a sensitive and specific ELISA for HEV, which is necessary to ellicidate its epidemiology. The most promising vaccine candidate to date has its origins in the virus isolated from stool specimens from this epidemic. Work continues on these specimens using polymerase chain reaction assays to measure virus excretioin in stool.

2. Bryan JP, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, Duncan J, Rafiqui AR, Malik IA, Purcell RH and LJ Legters. Pattern of anti-HEV by ELISA in an epidemic of hepatitis in Pakistan. Abstract presented at joint annual meeting of the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists. Atlanta, GA Oct 31 - Nov 4, 1993.

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4. Zhang HY, Caudill JD, Popkin TJ, Duncan JF, Hussain I, Ahmed A, Iqbal M, Malik IA and LJ Legters. Patterns of hepatitis E virus (HEV) excretion from selected patients in an outbreak. Abstract and presentation at the International Symposium on Viral Hepatitis and Liver Disease (8th Triennial Congress). Tokyo, Japan, May 10-14, 1993.

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This manuscript describes the pattern of arboviral exposure in this strategically important part of the world.

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Initial report of a hepatitis investigation in rural Belize

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Abstract

In spring 1991, Belizian health officials expressed concern about a possible hepatitis outbreak in a banana farming district. A study was designed to identify cases and to address the serological prevalence of hepatitis virus markers. Three populations were studied: (i) persons meeting a clinical case definition for hepatitis; (ii) designated banana workers; and (iii) people in a random sample of households in the community. Information was collected using questionnaires and sera were collected for laboratory testing. This report presents the preliminary results of a study conducted in June 1991. Among people who met the clinical case definition, 24% of 42 tested had immunoglobulin M antibody to hepatitis B virus (HBV) core antigen (anti-HBc IgM). In the worker and household survey populations, 284 and 280 people, respectively, were tested for anti-HBc IgM. In each group, 4% were positive. HBV surface antigen was found in 37% of 43 clinical cases, 18% of workers, and 13% of people in the household survey. Among the 3 study populations, the prevalence of HBV core antibody (anti-HBc) ranged from 73% to 81%. Almost all tested persons had evidence of prior hepatitis A virus infection. Evidence of prior infection with hepatitis viruses A and B was widespread, but an aetiology could not be established for most of the clinical cases. However, the prevalence of hepatitis B markers in this population was very high compared to other reports from the Caribbean.

Introduction

In May 1991, concern that cholera might be spreading north through Central America prompted officials of the Belizian Ministry of Health to initiate a cholera prevention and control campaign. In the Cowpen area of southeast Belize, there was no report of illness resembling cholera; however, banana farm workers and local health officials expressed concern about allegations that large numbers of people were or had been ill with hepatitis. Some people had diagnosed themselves as having hepatitis, based upon the occurrence of jaundice. In others, the diagnosis had been made by local health professionals.

Cowpen residents considered the appearance of hepatitis to be an annual occurrence associated with the dry season; they attributed the disease to poor water quality. However, many believed that the 1991 dry season had been more severe and prolonged than usual and that the number of hepatitis cases, particularly in young adults, was greater than that observed in past years. Concern heightened when a young pregnant woman allegedly died with hepatitis.

When the Belizian Ministry of Health requested assistance in investigating the alleged outbreak, we conducted a preliminary investigation in May 1991 to establish the cause(s) of the reported illnesses. The May investigation confirmed the presence of hepatitis in the Cowpen area, based upon abnormal liver function tests and serological studies for hepatitis virus markers. This was followed in June 1991 by an expanded effort to identify cases and to make a cross-sectional study for hepatitis markers in banana farm workers and their families. This is the initial report of the June 1991 investigation. Serological studies and data analyses are continuing, and results of work still in progress will be reported later.

Materials and Methods

Three study populations were defined: cases, banana workers, and households. Individual, household and farm questionnaires were developed in English and Spanish for the uniform collection of information. All individuals were given unique identifiers, which were assigned to serum collection tubes and questionnaires.

A case of hepatitis was defined as anyone who, in the preceding 6 months, based upon the review of a study team physician, had been diagnosed as having hepatitis by a health care worker or had experienced jaundice. Possible cases were identified for review by a team physician through (i) local health worker reports of currently or previously ill people, (ii) reports from farm and civic leaders of ill people, (iii) study team physicians holding sick call and making house calls on request, and (iv) study team members asking every adult contacted if they had been jaundiced or diagnosed as having hepatitis, or knew of anyone who had. After a study team physician determined that a person met the case definition, serum was collected and a case questionnaire was used to obtain illness data not covered in other questionnaires.

During May–June 1991, local officials in Cowpen estimated the total population of banana farm managers, banana field and shed workers, and family members to be 1300 to 1500 people. These people worked on 7 different banana farms and lived in 8 worker housing areas, all of which were identified for study. Farm personnel usually were studied at the farm packing shed and were questioned about demographic variables, the occurrence of illness, and variables associated with hepatitis virus transmission. An attempt was made to study all banana farm workers. However, 2 factors limited the percentage studied: (i) when banana harvesting and packing were occurring at high intensity, many workers could not be released to participate in the study; and (ii) when there was a lull in harvesting and packing, many workers immediately left the area to visit friends and relatives in Belize or in their homeland. At each household, a household census with demographic information was obtained, and questions were asked about the occurrence of illness and variables associated with hepatitis virus transmission.

Blood for serological studies was obtained from all consenting individuals over 4 years of age. Four years was established as the cut-off age because of reluctance on the part of most parents to have blood drawn from younger children. Response refusals were rare.

An attempt was made to test all sera for antibody to hepatitis A virus (anti-HAV), immunoglobulin M (IgM) antibody to hepatitis A virus (anti-HAV IgM), hepatitis B virus (HBV) surface antigen (HBsAg), antibody to HBV core antigen (anti-HBc), IgM antibody to HBV core antigen (anti-HBc IgM), and antibody to hepatitis C virus (anti-HCV). Only sera positive for HBsAg were tested for HBV e antigen (HBeAg) and antibody to hepatitis delta virus (anti-HDV). All clinical cases were tested for antibody to hepatitis E virus (anti-HEV). Sera

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from cases in which there was no serological evidence of infection with any of the hepatitis viruses also were tested by enzyme-linked immunosorbent assay (ELISA) for IgG and IgM antibodies against leptospira and 2 strains of hantavirus.

Anti-HAV, anti-HAV IgM, and anti-HDV were determined using ELISA kits from Abbott Laboratories (North Chicago, Illinois, USA). Testing associated with HBV also was done using Abbott ELISA kits. Ortho Diagnostics (Raritan, New Jersey, USA) ELISA kits were employed to determine the presence of antibody to HCV. Both the first generation anti-HCV tests and the second generation immunoblot assay for the detection of antibody to HCV (RIBA-HCV) were utilized. Antibody to HEV was detected using a prototype ELISA GOLDSMITH *et al.*, 1992; SKIDMORE *et al.*, 1992).

Data were entered into an EpiInfo database (DEAN *et al.*, 1990). Check files and double entry were used to ensure valid transfer of hard copy questionnaires to the data files. EpiInfo was also used to develop descriptive statistics.

Results

A summary of the serological testing results for hepatitis markers is presented in the Table. The clinical case, worker and household survey populations were not mutually exclusive.

77%) had anti-HBc, indicating prior HBV infection.

Of 482 inhabited dwellings in the 8 housing areas, 146 (30%) were randomly selected and studied. The household sample subgroup included 285 people. The mean age was 26 years (SD=15); 152 (53%) were males. Almost all (275, 98%) had anti-HAV. The 2 children with recent HAV infection described in the clinical case group are also shown in the household sample in the Table. Ten (4%) had anti-HBc IgM, and 37 (13%) had HBsAg. Twenty (54%) people with HBsAg also had HBeAg. As in the other subgroups, most (203, 73%) had evidence of past HBV infection.

In all population subgroups studied, only very small numbers of people were found to have had contact with HCV or HEV. There was no evidence of exposure to HDV or recent infection with leptospira or hantavirus.

Discussion

We found a high prevalence of people with evidence of prior infection with HAV and HBV, a high prevalence of HBsAg positivity, and evidence for recent HBV infection in 24% of the people with clinical illness. Overall, evidence of HBV infection was found in 73% of the people studied, and 16% were positive for HBsAg. The prevalence of anti-HCV was low, and no evidence was found for HDV infection.

Nearly all people studied had anti-HAV. In contrast,

Table. Summary of results of serological testing for hepatitis markers, Cowpen, Belize, 1991

Specific marker[a]	Clinical cases n=47 No. tested	Clinical cases n=47 No. positive	Banana workers n=290 No. tested	Banana workers n=290 No. positive	Household sample n=285 No. tested	Household sample n=285 No. positive
Anti-HAV	43	41 (95%)	284	277 (97%)	280	275 (98%)
Anti-HAV IgM	43	2 (5%)	282	0	278	2 (1%)
HBsAg	43	16 (37%)	284	50 (18%)	280	37 (13%)
HBeAg[b]	15	4 (27%)	49	13 (26%)	37	20 (54%)
Anti-HBc	42	34 (81%)	284	219 (77%)	278	203 (73%)
Anti-HBc IgM	42	10 (24%)	284	12 (4%)	280	10 (4%)
Anti-HCV	43	1 (2%)	284	3 (1%)	280	3 (1%)
Anti-HDV[b]	14	0	48	0	32	0
Anti-HEV[c]	38	2 (5%)	24	2 (8%)	18	0

[a]HAV=hepatitis A virus, IgM=immunoglobulin M, HBsAg=hepatitis B virus surface antigen, HBeAg=hepatitis B virus e antigen, HBc=hepatitis B virus core antigen, HCV=hepatitis C virus, HDV=hepatitis D virus, HEV=hepatitis E virus. Insufficient quantities of sera prevented complete testing of all people studied.
[b]Only people positive for HBsAg were tested. Expressed percentages are based upon the number of HBsAg positive people tested.
[c]Only clinical cases were tested. Some cases were workers; some were also part of the random household sample.

Forty-seven people met the clinical case definition of hepatitis. Their mean age was 26 years (SD=14); 29 (62%) were males. Twenty-nine cases were identified as workers, and 25 (86%) were male. The average age of worker cases was 32 years (SD=11).

Laboratory results were obtained for 43 cases. Two children were too young for drawing blood and 2 serum specimens were lost. Forty-one (95%) of the cases were positive for anti-HAV, and 2, both children, had anti-HAV IgM, indicating recent HAV infection. Ten (24%) were newly infected with HBV, as demonstrated by the presence of anti-HBc IgM, and 16 (37%) were HBsAg positive. Among those with HBsAg, 4 (27%) also had HBeAg. Evidence of past HBV infection (presence of anti-HBc) was found in 34 (81%).

A total of 512 male and 111 female banana workers was identified. Of these 623, 290 (46%) were studied and blood was tested from 284. In the subgroup of 290 workers, the mean age was 32 years (SD=13); 228 (79%) were males. There was no indication of recent HAV infection, but 277 (97%) had anti-HAV, indicating prior infection. Twelve workers (4%) had anti-HBc IgM and 50 (18%) had HBsAg. Among those with HBsAg, 13 (26%) also had HBeAg. The majority of workers (219,

anti-HEV could be demonstrated in only 2 cases and it was not possible to distinguish between recent and past infection. The study of sera from Cowpen for evidence of HEV infection is continuing because ELISA tests for anti-HEV are still in the early stages of development (GOLDSMITH *et al.*, 1992; SKIDMORE *et al.*, 1992); sensitivity and specificity have not been completely determined. Work is under way to identify any antigenic variation among hepatitis E viruses. Because we could not establish a serological diagnosis for most of our hepatitis cases, these might have been due to HEV or another agent of non-A, non-B hepatitis.

In the absence of a demonstrable viral agent, consideration must be given to toxic substances, including ethanol or a contaminated food supply. Alcohol consumption was noted but collection of these data was outside the scope of the present study. There was no indication of disruption in the food supply during the dry season to suggest that hungry people might have consumed grains or other foods heavily contaminated with fungi and fungal toxins. Epidemiological analysis of a vast amount of demographic data collected for people studied is in progress, with a wide range of possible aetiologies being considered.

HAV and HEV have faecal–oral routes of transmission and problems existed with both drinking water quality and sanitation in Cowpen. Our study indicated that hepatitis A in Cowpen is an illness of children. HEV may have been recently introduced, thus explaining at least some of the cases of non-A, non-B hepatitis in young adults. Prevention of both HAV and HEV infections is strongly dependent upon a purified water source, good personal hygiene, and proper disposal of human waste. After attention was directed toward the probability of enterically transmitted hepatitis virus or viruses in Cowpen, some farm managers and local inhabitants built improved water systems that included treatment measures.

In screening refugees to the USA from Asia, Africa and eastern Europe, investigators have reported HBsAg prevalences ranging from 1·1% to 15·5% (CRYSEL *et al.*, 1991). Along the southern border of Mexico, another study reported the seroprevalence of HBsAg to range from 4·2% to 17·3%. This prevalence is uniquely high among the reported prevalences from the Caribbean nations. Overall, 91% of that Mexican population had been infected with HBV, and HDV was present in 50% of those who were HBsAg positive (ALVAREZ-MUNOZ *et al.*, 1989).

Within Central America, the lowest reported prevalence of HBsAg (0·64%) was found in Costa Rica (SALOM *et al.*, 1990). Throughout the Americas, from 1975 to 1985, HBsAg prevalence was reported to range from 0·3% in the USA to 8·0% in Amazonia. The countries surrounding Belize have a reported HBsAg prevalence between 1% and 3% (FAY *et al.*, 1990). However, the true impact and prevalence of viral hepatitis throughout many countries in Central and south America have yet to be studied (PAHO, 1990; WHO TECHNICAL ADVISORY GROUP ON VIRAL HEPATITIS, 1988). Cowpen HBV marker prevalences match or exceed the highest 1980 prevalences reported in Senegal, Thailand, Uganda, Egypt, and India (SOBESLAVSKY, 1980).

In the Cowpen population, with a very high prevalence of HBV markers, we found little evidence of HCV infection. HBV and HCV are transmitted through direct contact with blood, blood products and possibly other body fluids. However, infection with HCV appears to occur independently from HBV and is a frequent complication of transfusions (HAYASHI *et al.*, 1991). HBV can be acquired as a result of intravenous drug use with shared needles, blood transfusion and sexual contact. Individuals infected with HBV who are positive for HBeAg are considered highly infectious. HBeAg was found in 26%, 27%, and 54% of those HBsAg positive within the three populations we studied.

The specific risk factors that made the Cowpen population particularly prone to HBV infection are still under study. We do have information that parenteral antimalarials, vitamins, antibiotics and intranvenous fluids were used without a physician's supervision, and that dental practices occurred outside a professional setting. Overall, people had a high level of interest in their health and the health of their family and friends. Generally recognized measures for preventing HBV infection include passive/active immunizations and counselling for behavioural modification. Efforts were initiated in 1991 in Belize to enhance the screening of blood donations for hepatitis markers. Screening of pregnant women for HBV infection must also be emphasized.

The high prevalence of HBV markers in the Cowpen population is also cause for concern about the potential for transmission of other blood-borne pathogens such as human immunodeficiency virus (HIV) and other retroviruses. Although human T cell lymphotropic viruses 1 and 2 have been found in 9% of one Indian tribe in Panama, it is not clear how transmission occurred (REEVES *et al.*, 1990). HIV infection is devastating the population in many of the same countries that reported high HBV rates 10 years ago. Greater knowledge about transmission characteristics is critical to the development of preventive measures against such agents.

Our study emphasizes the need to learn more about the transmission of hepatitis viruses in rural Belize in order to institute specific intervention efforts. It is essential to determine when and where infection occurred since the banana workers of Cowpen were not a stable population. Most people studied came from surrounding countries and lived in Belize for variable periods. Additionally, our cross-sectional study was done when there may have been an increase of hepatitis in the region. We may have looked at a rate of infection which reflected exposure in Belize, outside Belize, or both.

In summary, we found a high prevalence of HBV infection markers and an unusually high prevalence of HBsAg positivity in Cowpen, Belize. Additionally, almost all people had evidence of HAV infection by the time adulthood was reached. Both hepatitis A and B are preventable through well established environmental controls and personal behavioural changes. Detailed descriptive epidemiological studies followed by intervention initiatives are needed to reduce hepatitis virus transmission and the associated morbidity.

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THE PREVALENCE OF HEPATITIS A, B AND C INFECTION AMONG DIFFERENT ETHNIC GROUPS IN BELIZE

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Abstract. Little is known about the prevalence of infection with hepatitis viruses in Belize, Central America. We conducted a serologic survey among members of the Belize Defence Force (BDF), which is composed of the five major ethnic groups in Belize, to estimate prevalence rates of hepatitis A, B, and C among military-aged men and women in Belize. Of approximately 600 men and women in the BDF, 492 (82%) completed a questionnaire and blood collection. Antibody to hepatitis A was found in 94%, with similar rates by age, sex, rank, and ethnicity. Antibody to hepatitis B core antigen (anti-HBc) was found in 31%. Rates of anti-HBc varied significantly among the ethnic groups with the lowest rates in Mestizo (5%) and Mayan Indians (9%), and significantly higher rates among Creoles (30%) and Garifuna (56%). Rates increased with increasing age from 28% in those 18–24 years old to 35% in those ≥ 35 years old ($P = 0.07$, by chi-square test for trend). Hepatitis B surface antigen was found in 21 (4%) overall. Antibody to hepatitis C was found in two (0.4%). In this young healthy population, exposure to hepatitis A before the age of 18 is almost universal, while exposure to hepatitis B is related to age and ethnic origin.

There have been no reports of the prevalence of viral hepatitis for the Central American country of Belize. The only serologic studies of hepatitis virus markers in Belize are from a recent outbreak investigation in a community of immigrant banana farm workers.[1] The following study was conducted during the summer of 1992 to determine the prevalence of hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infection in a Belizean population, the Belize Defence Force (BDF).

The BDF is a light-infantry battalion, supported by air and maritime wings. Formed in 1978, the combined strength of active duty personnel is approximately 600. Since members of the BDF are recruited from five diverse ethnic groups from all six districts of the country, the survey was done to obtain information about the prevalence of hepatitis A, B, and C in these ethnic groups, and to determine the need for preventive intervention in this military organization.

MATERIALS AND METHODS

The study was approved by a Human Use Review Committee of the Ministry of Health of Belize, the Defence Board in Belize, and the Human Use Review Committee at the Uniformed Services University of the Health Sciences. All available personnel of the BDF were invited to participate in the study. After obtaining informed consent from each volunteer, questionnaire data (which included identifying demographic, behavioral, and medical history information) was collected by interviewers. A 12-ml venous blood sample was then collected and labeled with a unique identification number. Only the principal investigator had access to the code linking the number to the volunteer's questionnaire.

Serum samples were then tested by enzyme-linked immunosorbent assays (ELISAs) for antibody to HAV (anti-HAV) (Hepanostika® anti-HAV), hepatitis B surface antigen (HBsAg) (NML® ELISA HBsAg), and antibody to HBV core antigen (anti-HBc) (Hepanostika® ANTI-CORE) (all from Organon Teknika Corporation, Durham, NC). Testing for anti-HCV was done with an anti-HCV second generation enzyme immunoassay (Abbott Diagnostics, Abbott Park, IL). All tests with positive results were repeated at least twice for confirmation.

RESULTS

Study population

There were 519 subjects, which represented 87% of eligible BDF members, entered into the

TABLE 1

*Proportion of ethnic groups in the population of Belize and the Belize Defence Force (BDF)**

	% of population	% of BDF
Creole	29.8	33.0
Garifuna	6.6	33.0
Mestizo	43.6	18.3
Mopan Maya	3.7	9.7
Ketchi Maya	4.3	1.5
East Indian	3.5	1.6
Other	8.5	3.0

* The total population of Belize in 1991 was 184,722 and the BDF had 600 members.

study. Questionnaire data was available on 492 (95%) of these. These 492 subjects comprise the study population. Of the 472 males and 20 females, there were 455 (92%) enlisted personnel, 35 officers (80% of all officers of the BDF) and two civilians. The age range was 18–44 years, with the mean age of the enlisted personnel and officers being 25 years and 29.6 years, respectively.

The racial/ethnic composition of the study population of the BDF in comparison with the total population of the country of Belize is shown in Table 1. While the Creole component of the BDF comprised a similar proportion to that of the general population of Belize, the Garifuna group comprised a greater proportion and the Mestizo a lesser proportion in the BDF than in the general population. This survey was also not representative of the indigenous people of Belize (the Mopan and Ketchi Maya). Whereas the Mopan Maya predominantly live in three districts in the country, almost all (92%) of those in the survey were from the southern district of Toledo, and 50% were from a single village. The Mopan Maya were over-represented in the BDF while the six Ketchi Maya were under-represented.

Hepatitis A prevalence

Almost all of the study participants had evidence of prior HAV infection (94% were reactive to anti-HAV). There was no difference in prevalence of anti-HAV by sex, ethnic group, officers versus enlisted personnel, or age group (Table 2). Only 18 (4%) of the 464 with anti-HAV reported a history of jaundice; 10 of these 18 also had serologic evidence of previous infection with HBV.

TABLE 2

*Hepatitis A (HAV) and B seroprevalence in Belize Defence Force personnel, by selected demographic characteristics**

	No. tested	% anti-HAV reactive	% anti-HBc reactive
Total study population	492	94	33
Male	472	94	31
Female	20	100	45
Rank			
Enlisted	455	95	32
Officer	35	91	29
Civilian	2	100	0
Ethnicity			
Garifuna	163	98	56
Creole	162	90	30
Mestizo	90	96	5
Maya Mopan	48	100	10
Ketchi Maya	6	83	0
East Indian	8	88	13
Other	15	87	33
Age group (years)			
18–24	247	95	28
25–29	130	92	32
30–34	75	95	40
≥35	40	98	35

* Anti-HBc = antibody to hepatitis B virus core antigen.

Hepatitis B prevalence

One hundred twenty-one subjects (33%) were reactive for anti-HBc and 21 (4%) had HBsAg. The combined Ketchi and Mopan Mayan component of the BDF had a 9% prevalence for anti-HBc. The Mestizo (mixed Mayan and Spanish) ethnic group had a 5% prevalence for anti-HBc. For the Creole group (of mixed African ancestry), the prevalence of anti-HBc (30%) was significantly higher than in the Mestizo or Maya ($P < 0.001$). Anti-HBc was found most commonly in the Garifuna component of the BDF (56%), a significantly higher percentage than for the Creole group ($P < 0.001$) (Table 2). In addition, a higher proportion of the Garifuna with anti-HBc were positive for HBsAg (19 [21%] of 91) compared with two (3%) of 49 of the anti-HBc–positive Creole population ($P < 0.01$).

The prevalence of anti-HBc generally increased with age, from 28% in subjects 18–24 years old to 40% in those 30–34 years old, but the prevalence was only 35% in those > 35 years ($P = 0.07$, by chi-square test for linear trend). Compared with the prevalence of anti-HBc in Creoles, which showed a general increase with

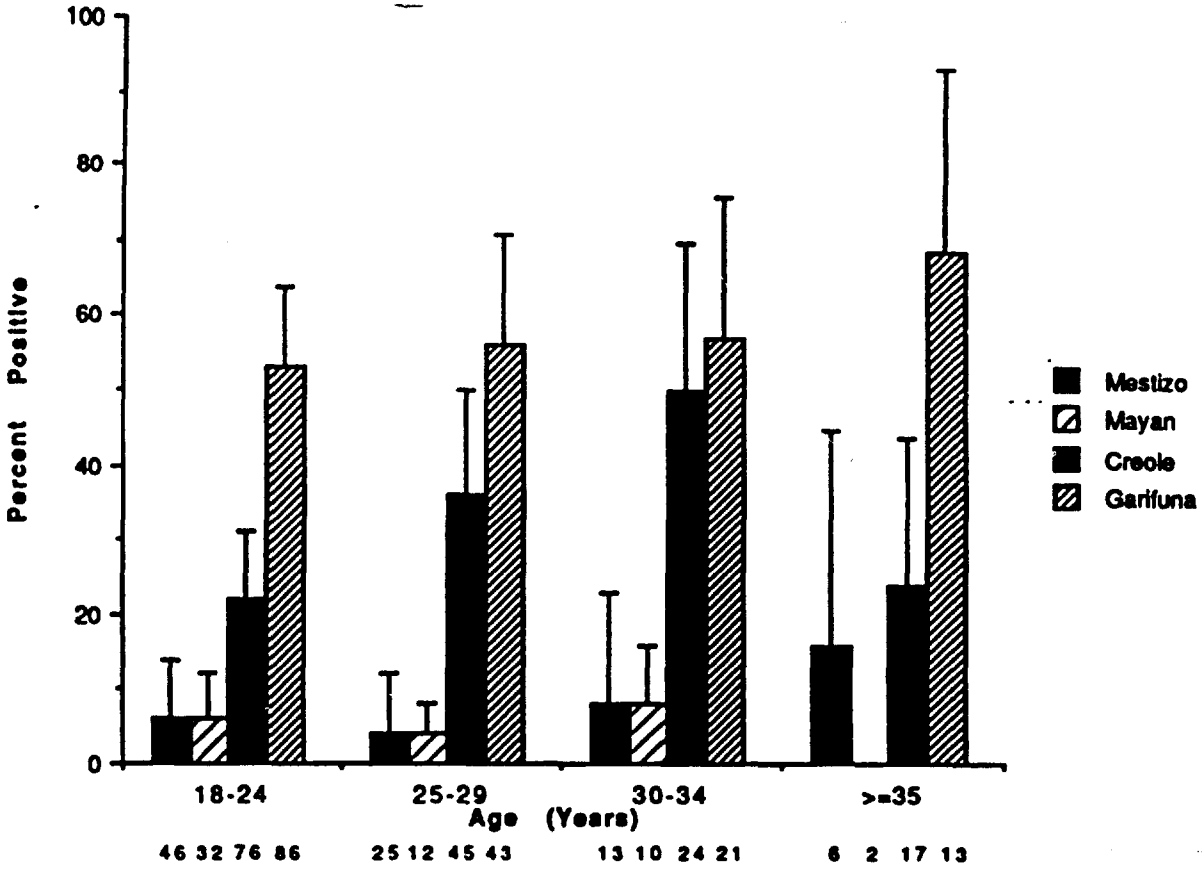

FIGURE 1. Proportion of the Belize Defence Force with antibodies to hepatitis B virus core antigen (anti-HBc), by age and ethnic groups. Bars indicate the upper 95% confidence interval. Values below the ages indicate the number studied in each group.

age from 22% in subjects 18–24 years old to 50% in subjects 30–34 years old ($P = 0.15$, by chi-square test for linear trend), the prevalence of anti-HBc in the Garifuna component of the BDF remained stable (53–57%) in those 18–34 years old (Figure 1). The prevalence of anti-HBc was not significantly different among the seven enlisted ranks ($P = 0.2$) and did not significantly correlate with years of service in the BDF ($P = 0.09$).

Risk factors known to be related to HBV transmission and included in the study are shown in Table 3. Anti-HBc was more commonly found in those with a history of pierced earlobe(s) (38% versus 30%; $P = 0.07$). Those with both pierced ears and tattoos had a higher prevalence of anti-HBc (40%) compared with those who had neither (31%; $P = 0.18$). However, there were no statistically significant associations between the risk factors listed and anti-HBc reactivity. Because of issues of confidentiality, information about sexual practices potentially related to HBV transmission was not included in the questionnaire.

Hepatitis C prevalence

Only two (0.4%) of 492 subjects had repeatedly reactive anti-HCV. One of the cases was also reactive for anti-HBc. Neither subject gave a history of hepatitis, receipt of blood transfusions, nor ear piercing. One had a tattoo.

DISCUSSION

In this study, there was a high prevalence of antibody to HAV (94%), with a low rate of a history of jaundice, suggesting asymptomatic infections acquired early in life. This pattern of HAV seroreactivity is consistent with findings in other economically developing countries where infection occurs primarily in young children, and

almost all have antibody to HAV by the age of 10 years.[2]

Evidence of HBV infection was found in 33% of the BDF personnel studied and 4% were positive for HBsAg. In the previously mentioned study of an outbreak investigation of hepatitis in rural Belize, evidence of HBV infection was found in 77% of workers on a banana farm and 18% were positive for HBsAg.[1] In that study, the sample population consisted mainly of refugees from the neighboring Central American Republics of El Salvador and Guatemala. No individuals of refugee status were included in our study population.

The results suggest that HBV infection is concentrated in a small segment of the population of Belize. The Garifuna are descendants of African slaves and Carib Indians who settled in the Bay Islands of Honduras and in coastal settlements of the southern districts of Stann Creek and Toledo in Belize about 1800. The prevalence of HBV in this ethnic group in the BDF was several times that of the combined indigenous Maya component or the Mestizo group, and almost twice that of the Creole population.

The relatively stable prevalence across all age groups for the Garifuna component of the BDF may indicate perinatal and/or pre-adolescent (horizontal) transmission of HBV such as that which occurs among nations of Southeast Asia and the Pacific basin, and in some countries in Africa and South America.[3–5] The fact that a relatively large proportion (21%) of the Garifuna with anti-HBc had HBsAg also suggests that infection may be occurring at an early age with resultant chronic carriage of HBsAg.

In contrast, the prevalence of anti-HBc in the Creole population increases markedly across the various age groups, rising from 22% in those 18–24 years old to 50% in those 30–34 years old, suggesting infection acquired in adulthood. This may indicate modes of transmission similar to those in North America and Western Europe, in which HBV infection is spread mainly through sexual contact and/or intravenous drug abuse.[3, 6, 7] It is our observation that intravenous drug abuse is uncommon in the BDF. The human immunodeficiency virus, which shares modes of transmission with HBV, has not been found in more than four years of mandatory screening of BDF recruits including those in the present study. Thus, modes of transmission of HBV in Creoles requires additional study.

TABLE 3

*Potential risk factors for hepatitis B in the study population**

	No. anti-HBc reactive/no. tested	% reactive	Odds ratio	P
Blood transfusions				
Yes	5/15	33		
No	151/477	32	0.8	0.9
History of hepatitis				
Yes	4/10	40		
No	152/482	32	1.4	0.6
Tatoos				
Yes	91/286	32		
No	65/206	32	1.0	0.95
Ears pierced				
Yes	45/117	38		
No	111/375	30	1.5	0.07

* Anti-HBc = antibody to hepatitis B virus core antigen.

Other populations with Carib Indian or West African ancestry have previously been noted to have high rates of hepatitis B. An investigation of an epidemic of severe hepatitis that occurred among the Yucpa Indians of Venezuela, who are of Carib Indian ancestry,[8] revealed a high prevalence of anti-HBc (94%) and HBsAg (64%). Evidence of infection with the delta viral agent (HDV) was found in 86% of those with HBsAg.[9] Hepatitis B is endemic in West Africa, the origin of the majority of slaves who were transported to the Caribbean. In Senegal, 80% of the population is infected with HBV by seven years of age.[5]

Another interesting observation is the low prevalence of anti-HBc (9%) in the indigenous people of Mayan descent in the BDF. This contrasts with high prevalence in other indigenous populations of the Americas (Alaskan Eskimos, American Indians, and Guatemalan refugee camps in southeast Chiapas, Mexico).[10, 11]

The potential risk for infection with HDV, as occurred in the Yucpa Indians of Venezuela and the study population in Chiapas, Mexico,[9, 11] also exists for the Garifuna. This is in addition to the enhanced risk of serious sequelae associated with chronic HBV infection such as chronic active hepatitis, cirrhosis, liver failure, and primary hepatocellular carcinoma.[12] No evidence of HDV infection was found in the HBsAg carriers identified in the outbreak investigation of hepatitis in southern Belize.[1]

Since the BDF sample population is restricted

to individuals ≥ 18 years of age, it is recommended that a further cross-sectional study be done of preschool and school-aged children in the Stann Creek district, where approximately half of the Garifuna population of Belize lives. If most infections occur during infancy or childhood, an aggressive program of immunization aimed at young children could result in a substantial decrease in the prevalence of HBV in the Garifuna community, as has been seen in The Gambia.[5] In that country, vaccination reduced the prevalence of HBV infection in immunized children from 41% to 5% in one village and from 76% to 19% in another. A strategy for reducing transmission in soldiers would include education and vaccination.

In the interim, screening of pregnant Belizean women and blood donors is of paramount importance. Such prenatal testing for HBsAg should identify at-risk newborns who require immunoprophylaxis and vaccination for the prevention of perinatal infection as a component of a comprehensive strategy to control HBV transmission in Belize.[13, 14]

Antibody to HCV was uncommon in this study population (0.4%). Since hepatitis C is transmitted mainly through intravenous drug use or blood transfusions, this low prevalence is consistent with the low prevalence of observed intravenous drug use in the BDF. The low prevalence further indicates that this population with a high prevalence of anti-HBc may continue to donate blood with minimal risk of transmitting hepatitis C.

Disclaimer: The opinions and assertions contained in this article are not to be considered official or to necessarily represent the views of the Uniformed Services University of the Health Sciences.

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Enclosure 3

SCIENCE, SKILLS AND STRATEGIES

PREVENTION 94

ABSTRACT FORM

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PREVALENCE OF HEPATITIS B MARKERS AMONG WOMEN ATTENDING SELECTED PRENATAL CLINICS IN BELIZE, CENTRAL AMERICA

Alicia Scott-Wright, MD MPH; J. Bryan, MD; J. Gaydos, MD MPH, Uniformed Services Univ. of the Health Sci: S. Hakre; R. Jaramillo; L. Reyes, Epidemiol. Research Center, Belize.

We initiated hepatitis B (HB) screening of women attending prenatal clinics in Belize. Risk factors for HB infection and demographic data were obtained by interview. Of 548 women, 81 (15%) were positive for HB core antibody (anti-HBc); HBsAg was detected in only one woman. Rates varied significantly by district (range 0%-44%) and ethnicity (Creole, 19%, East Indian, 6%, Garifuna, 41%, Mayan, 22%, and Mestizo, 9%). Identified risks were: number of people in home (p=.004), number of sex partners (p=.02), and age at first intercourse (p=.04). Reactive syphilis serology, tattoos, IV drug use, and transfusions were not identified risks. Highly variable differences in anti-HBc rates by district and ethnicity may permit the targeting of public health resources for HB education and prevention programs. Strategies to immunize at-risk infants and prenatal women may be effective in interrupting HB transmission.

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February 1, 1994

Randomized Comparison of 5 and 10 μg Doses of Two Recombinant Hepatitis B Vaccines.

Joe P. Bryan, MD; Peter G. Craig, MBBS, MPH; Linda Reyes; Shilpa Hakre; Ruth Jaramillo; Harold Harlan, PhD; Philip MacArthy, PhD; and Llewellyn J. Legters, MD, MPH.

Uniformed Services University of the Health Sciences, Bethesda, MD;

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Enclosure 4

Disclaimer

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Abstract

The high cost of hepatitis B vaccines remains an obstacle to their use. Since the recommended adult dose of Recombivax HB (MSD) is 10 μg and that of Engerix B (SKB) is 20 μg, we sought to determine if 10-μg doses of each vaccine are equally immunogenic. Further, since 5-μg doses of Recombivax are routinely used in those ≤ 29 years of age in the US military, we sought to compare this dose with 5-μg doses of Engerix B.

Methods: Members of the Belize Defence Force who were ≥ 18 years of age (median 24) without detectable anti-HBc were randomly assigned to receive Recombivax, 5 or 10 μg, or Engerix, 5 or 10 μg IM at 0, 1, & 6 months. Randomization was weighted toward Engerix.

Results: After 3 doses, the highest concentrations of anti-HBs were among those receiving Recombivax 10 μg (n = 22) or 5 μg (n = 46). The geometric mean anti-HBs concentrations (Geo mean) were 744 and 570 IU/ml, respectively. Similar proportions in the two groups developed ≥ 10 IU/mL anti-HBs (100% and 98%). Less favorable results were observed among the 91 persons who received Engerix 10 μg: Geo. mean anti-HBs, 325 IU/L; 91% ≥ 10 IU/L. The 87 persons who received Engerix 5 μg had the lowest Geo. mean, 177 IU/L ($p < 0.05$). This group had a lower rate ≥ 10 IU/l, 86% ($p > 0.05$) and ≥ 100 IU/l, 63% ($p < 0.05$) than those in either Recombivax regimen.

Conclusions: The 4 regimens resulted in rates of anti-HBs ≥ 10 IU/L which were not statistically different. In this young, healthy population, half doses of Recombivax and Engerix are highly immunogenic and may result in significant vaccine cost savings.

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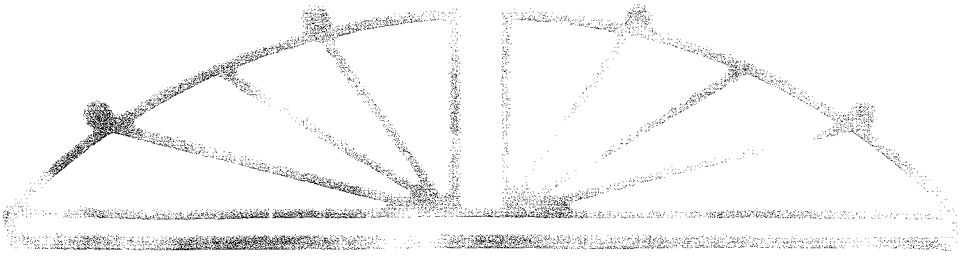

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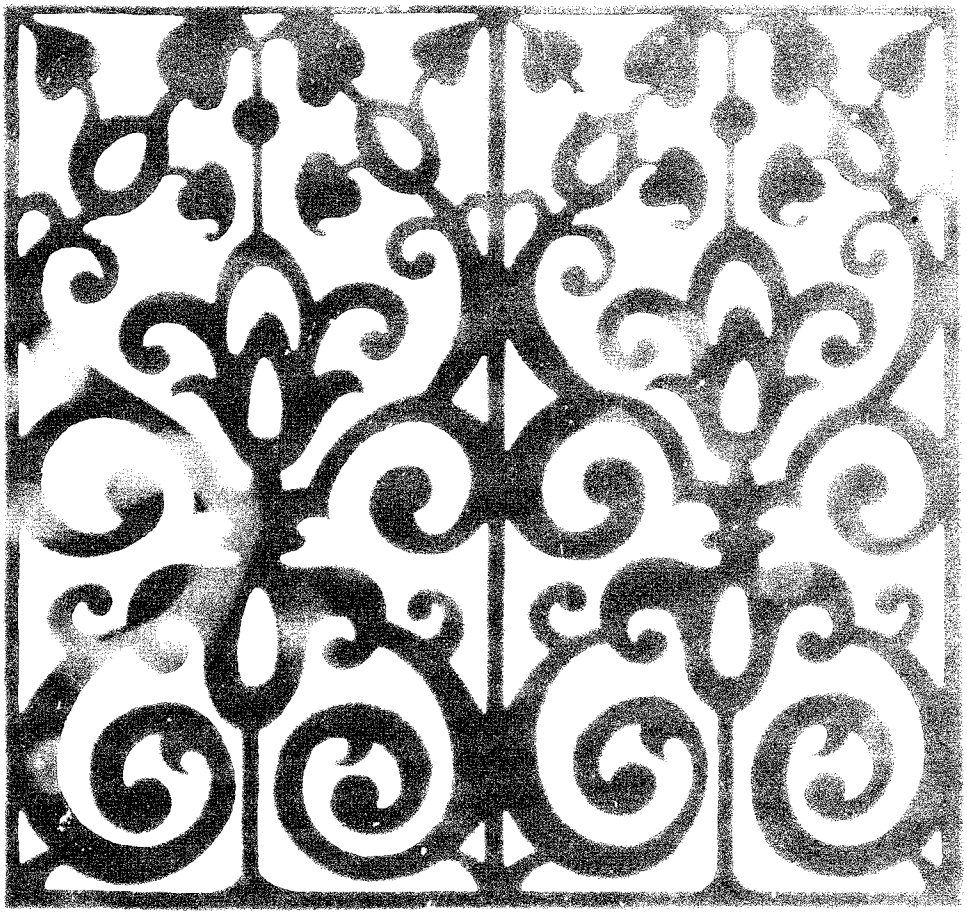

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Randomized Comparison of 5 and 10 μg Doses of Two Recombinant Hepatitis B Vaccines. J. BRYAN*, P. CRAIG, P. MACARTHY, L. REYES, S. HAKRE, R. JARAMILLO, & L. LEGTERS. Uniformed Services University of the Health Sciences, Bethesda, MD; Belize Defence Force, Belize; Walter Reed Army Institute of Research, Washington, DC; and Belize-USUHS Epidemiologic Research Center, Belize.

The high cost of hepatitis B vaccines remains an obstacle to their use. Since the recommended adult dose of Recombivax HB (MSD) is 10 μg and that of Engerix B (SKB)is 20 μg/ml, we sought to determine if 10 μg doses of each vaccine are equally immunogenic. Further, since 5 μg doses of Recombivax are routinely used in those ≤ 30 years of age in the US military, we sought to compare this dose with 5 μg doses of Engerix B.

Meth: Members of the Belize Defence Force who were ≥ 18 years of age (median 24) who tested negative for anti-HBc were randomly assigned to receive Recombivax HB, 5 or 10 μg, or Engerix B, 5 or 10 μg IM at 0, 1, & 6 m. Randomization was weighted to Engerix B.

Results 1 mo. after 2 doses follow:

	Recomb 5 μg	Engerix 5 μg	Recomb 10 μg	Engerix 10μg
Number	57	100	25	106
% ≥0.1 mIU/ml	87%	60%	92%	68%
% ≥ 10 mIU/ml	76%	33%	60%	43%
Mean anti-HBs	52	23	51	44

Conc: Recombivax HB in 5 μg doses appears to give results similar to 10 μg doses in these young adults. Similar doses of Engerix B produced significantly lower rates of anti-HBs than Recombivax.

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Antibody to Trypanosoma cruzi in Three Populations in Belize.

R. JARAMILLO*, I. GABOUREL, J. BRYAN, J. SCHUR, A. PAN.
Epidemiologic Research Center and the Ministry of Health, Belize City, Belize, The Uniformed Services Univ. of the Health Sciences, Bethesda, MD and Abbott Laboratories, North Chicago, IL.

A cross sectional study was conducted to determine the prevalence of antibody to Trypanosoma cruzi, the agent of Chagas' disease among three population in Belize. Specimens were tested using the ABBOTT CHAGAS ANTIBODY EIA (2.0 GEN). Confirmatory testing with three single antigen EIAs and radioimmunoprecipitation assay (RIPA) was performed. Four (0.85%) of 470 blood donors at the Belize City Hospital were reactive including 2 (8%) of 25 from countries known to be endemic for Chagas' disease. Among 467 healthy members of the Belize Defense Force, none were reactive. The final group included workers and families primarily from other Central American countries at a banana plantation in a rural area of the country. Twenty-six (5.9%) of 442 sera were reactive. The age prevalence was 5.3% of 75 <14 years, 4.2% of 236 15 to 34 years, and 9.7% of 124 ≥35 years. The prevalence was similar in males (6.7% of 280) and females (5.8% of 154). The prevalence in those born in Belize 4/56 (7.1%) was similar to that of those born in El Salvador 9/110 (8.2%), Guatemala 5/117 (4.3%) and Honduras 8/129 (6.2%). Of the 4 persons with reactive sera who were born in Belize, the immigrant mother of one was also reactive suggesting possible congenital transmission. Studies of 30 of the above reactive sera confirmed antibody to T. cruzi antigens in 20 by confirmatory EIA and in 28 by RIPA. Additional studies should focus on the epidemiology of T. cruzi and ways to reduce risk of transfusion related infections in Belize.

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Trypanosoma cruzi | Chagas' Disease | Belize

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proportion of males. During 1990, the proportion of gravid *P. papatasi* increased progressively during the five month period of May-September and averaged 29.5% for interior and 29.7% for exterior collections. Although monthly density of *P. papatasi* was frequently greater during 1991, proportions of gravid flies were significantly lower in each month and averaged 14.9% for interior and 12.3% for exterior collections.

233 CHAGAS' DISEASE IN THE UNITED STATES. A DIAGNOSTIC TEST DETERMINING THE PREVALENCE OF SEROREACTIVE ANTIBODIES TO *TRYPANOSOMA CRUZI* IN BLOOD DONORS. Pan AA*, Brashear RJ, Schur JD, Winkler MA, Shih J, and Decker R. Transfusion Diagnostics, Abbott Laboratories, North Chicago, IL.

Several recent incidents of transmission of Chagas' disease through transfusion of blood in the United States have increased our awareness of this threat to the blood supply. We have enhanced a second generation Chagas Antibody Enzyme Immunoassay (EIA) to a sensitivity of >99.99% and a specificity >99.98%. The prevalence of antibodies to *Trypanosoma cruzi* on 13,309 U.S. sera (7835 hispanic surnames; 5475 non-hispanic surnames) has been tested with this EIA. Consensus positive samples by IFA and HA from Argentina have also been assayed. A confirmatory scheme to assess the seropositivity of repeat reactive samples was developed involving three separate purified antigens in an EIA, and using iodinated antigens in a radioimmunoprecipitation assay (RIPA) format. The results showed thirteen sera were confirmed positive for a prevalence rate of 0.166% in the U.S. hispanic surname population. The use of questionnaires in the U.S. based on geographic origin or high risk factors would be discriminatory and, therefore, be unsuitable. This kind of deferral would also eliminate valuable non-infected blood donors, limit the number of autologous transfusions, and reduce the number of organ or marrow transplants. Our studies indicate that even with the current questionnaires in place and the current serological tests for blood borne pathogens (e.g., HIV, HTLV-I, and hepatitis), a rate of one in 603 exists for hispanic-surname donors with antibodies to this parasite.

234 EVALUATION OF A SHORT-COURSE (TEN DAYS) HIGH-DOSE GLUCANTIME IN THE TREATMENT OF CUTANEOUS LEISHMANIASIS IN GUATEMALA. Arana BA*, Navin TR, Arana FE, Berman JD. Universidad de Calle, Guatemala City, Guatemala; Division of Parasitic Diseases, Centers for Disease Control, Atlanta, GA; Walter Reed Army Institute of Research, Washington, DC.

To determine if the traditionally recommended 20-day treatment course of Glucantime for cutaneous leishmaniasis could be shortened without loss of efficacy, a comparative trial was conducted in which 44 Guatemalan men with parasitologically confirmed cutaneous leishmaniasis were randomly divided into two treatment groups: Glucantime (20 mg/kg) given intravenously each day for 20 days; and the same daily dose of Glucantime for 10 days. Both treatment groups were associated with similar response rates; 21 (95%) of the 22 patients in each group were clinically cured by 13 weeks after the initiation of treatment, and test-of-cure cultures at 9 weeks were negative. These preliminary data suggest that the traditionally recommend 20-day course of Glucantime for the treatment of cutaneous leishmaniasis may be significantly shortened without loss of efficacy.

235 SEROLOGICAL SCREENING FOR *TRYPANOSOMA CRUZI* AMONG CHILDREN IN CENTRAL BRAZIL. Andrade ALSS, Zicker F., Luquetti AO, and Martelli CMT. Institute of Tropical Pathology and Public Health, Federal University of Goias, Brazil; and Pan American Health Organization, Maracay, Venezuela.

The assessment of the impact of the programs for Chagas' disease control is usually based on epidemiological monitoring of house infestation and eventually by serological evaluation of selected cohorts of the population. In this study we report the result of a cross-sectional survey conducted

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PRELIMINARY OBSERVATIONS ON THE CHANGING ROLES OF MALARIA VECTORS IN SOUTHERN BELIZE[1]

D. R. ROBERTS,[2] O. CHAN,[3] J. PECOR,[4] E. REJMANKOVA,[5]
S. MANGUIN,[2] J. POLANCO[3] AND L. J. LEGTERS[2]

ABSTRACT. A survey for larval and adult *Anopheles* mosquitoes was conducted in Toledo District of southern Belize during August–September 1992. We surveyed for larvae in 145 habitats and conducted paired indoor–outdoor collections of adult mosquitoes landing on humans at 6 houses. In 1940–41, Kumm and Ram reported *Anopheles darlingi* females to be the most common *Anopheles* mosquitoes inside houses and reported no specimens of *Anopheles vestitipennis* in southern Belize. In our 1992 survey we found no *An. darlingi* mosquitoes either as adults or larvae. More *An. vestitipennis* females were captured indoors than outdoors, whereas most *Anopheles albimanus* and *Anopheles apicimacula* females were captured outdoors. All 3 species were represented occasionally in 145 collections of larvae from diverse habitats. *Anopheles vestitipennis* now appears to be a potentially important vector of malaria during the wet season in Toledo District.

The presence of *Anopheles darlingi* Root in Belize was first reported by Komp in 1940. The identity of Komp's original *An. darlingi* specimens was recently verified by Linthicum (1988). In 1941, Kumm and Ram documented the occurrence of house-frequenting populations of *An. darlingi* in the Toledo and Stann Creek districts of Belize (Fig. 1). Kumm and Ram relied heavily on searches of houses for resting mosquitoes as their primary survey method. *Anopheles darlingi* was found in 3 of 7 localities surveyed in Toledo District and in 5 of 7 localities in Stann Creek District. The larvae of *An. darlingi* were also collected in both districts. *Anopheles vestitipennis* Dyar and Knab was not collected as adults or larvae in Toledo District, but was collected as adults at 3 of 7 localities and as larvae in Stann Creek District. *Anopheles albimanus* Wiedemann was the most widely distributed species, being present at 9 of 14 localities from both districts. Larvae of *An. albimanus* were also collected in both Toledo and Stann Creek districts. Malaria sporozoites were found in the salivary glands of *An. darlingi* and *An. vestitipennis*, but not *An. albimanus*. *Anopheles darlingi* was the dominant indoor anopheline, representing more than 70% of the anophelines caught indoors in rural areas.

The Kumm and Ram survey was conducted more than 50 years ago, prior to the use of DDT in the malaria control program in Belize. Since their survey, no comparable data have been published for the Toledo District. Thirty years later, Bertram (1971) did not encounter a single specimen of *An. darlingi* in an extensive survey of adult mosquitoes in northern Belize, including areas of Stann Creek District. Although few collections have been conducted in Belize, the last documented appearance of *An. darlingi* in Belize was at Serra de Aqua in June 1946 (Linthicum 1988).

We initiated a malaria vector research program in Belize in 1990 and conducted extensive larval surveys in northern Belize, including Corozal, Orange Walk, Belize City, Cayo, and Stann Creek districts. No *An. darlingi* or *An. vestitipennis* larvae were collected in those surveys (Rejmankova et al. 1993). In a recent wet season survey in Toledo District we included nighttime, paired indoor–outdoor landing collections from humans to increase the likelihood of detecting the presence of *An. darlingi* and *An. vestitipennis*. These collections were performed by capturing mosquitoes as they landed on the exposed legs and feet of 2–4 collectors. Paired indoor–outdoor collections, using 1–2 collectors per indoor or outdoor site, were conducted one evening at each of 6 localities from 1830 to 1915 h. Based on past experience (Roberts et al. 1987), we expected the sunset interval to be a period of peak *An. darlingi* host-seeking activity. After completing the survey we learned that Rivera-Nunez (1990[6]) recently reported a sunset peak (1800–

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[6] Rivera-Nunez, L. A. 1990. Algunos aspectos de comportamiento de *Anopheles darlingi* (Diptera: Culicidae) de la Ceiba, Atlantida, Honduras. Thesis. Maestria en Entomologia. Universidad de Panama, Panama City, Panama.

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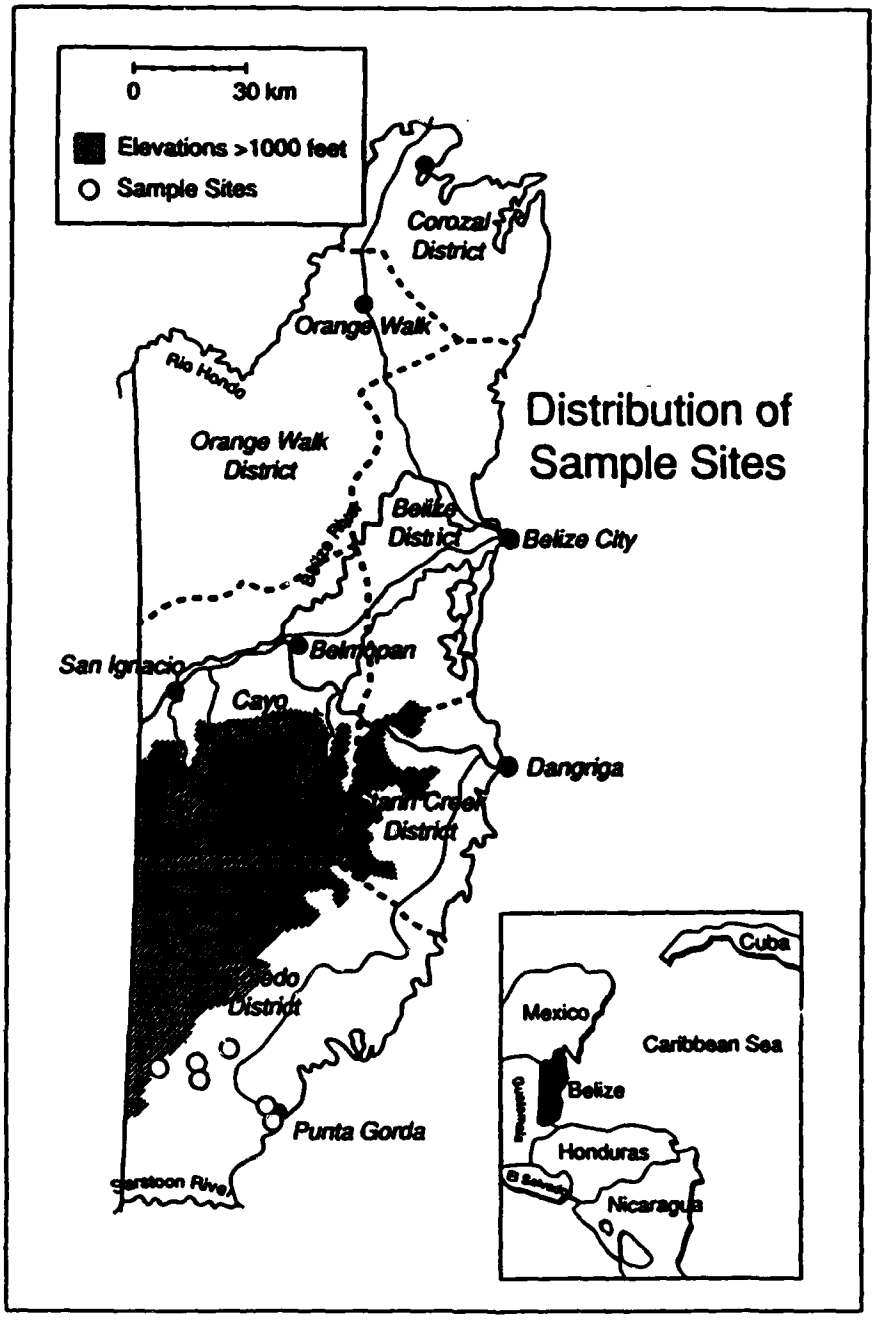

Fig. 1. Map of Belize depicting the distribution of collection sites in Toledo district, near Punta Gorda, Belize.

1900 h) in activity for *An. darlingi* populations in Honduras. Although we employed uniform collecting methods, we could not control for the numbers of children and adults who gathered around the collectors at both indoor and outdoor collecting sites. Although the collection data were not strongly quantitative, observations on relative composition of indoor versus outdoor collections seemed valid. Most houses had dirt floors and were constructed with loose-fitting wood or

Table 1. Numbers of anophelines collected from humans in paired indoor–outdoor landing collections in the Punta Gorda area of southern Belize during August and September 1992.

	Anopheles albimanus	*Anopheles vestitipennis*	*Anopheles apicimacula*
Collection site	Inside/outside	Inside/outside	Inside/outside
Jacinto Landing	2/3	27/4	0/0
Santa Helena	2/1	0/0	0/2
Crique Mafredi[1]	5/2	39/7	7/29
Crique Trosa[1]	0/2	9/0	9/33
Punta Gorda	0/2	2/1	0/0
Big Fall	1/25	0/1	0/0
Totals	10/35	77/13	16/64

[1] Houses not sprayed with DDT.

palm slats and a thatch roof. Two houses in Punta Gorda and Big Fall were solid and tightly enclosed.

Collections were performed at 6 sites in the area of Punta Gorda (Toledo District) in southern Belize (Fig. 1). Although collections were conducted in the same general areas surveyed by Kumm and Ram (1941), due to demographic changes during the intervening 51 years, their specific sites were no longer in existence. In addition to the nighttime landing collections, we conducted larval collections at 145 sites in Toledo District. Specimens from all categories of collections have been deposited in the mosquito collection of the Walter Reed Biosystematics Unit at the Smithsonian Institution.

No larvae or adults of *An. darlingi* were encountered during our survey. The dominant species collected biting indoors was *An. vestitipennis* (Table 1). Both *an. albimanus* and *An. vestitipennis* were widely distributed, being present at 6 and 5 sites, respectively. Overall, larger proportions of *An. albimanus* (78%) and *Anopheles apicimacula* Dyar and Knab (80%) were collected outdoors than indoors. In contrast, 86% of all *An. vestitipennis* females were collected indoors. All 3 *Anopheles* species collected in landing captures were also represented in the larval collections.

Collection data presented herein indicate that *An. darlingi* is either restricted to specific localities that we did not sample, very uncommon, or possibly absent in Toledo District. In April and May 1993, we finally encountered populations of *An. darlingi* adults in riverine environments of Stann Creek District. As a consequence, we think *An. darlingi* is probably present in Toledo District, but is limited to specific riverine localities.

Although *An. vestitipennis* was not encountered in Toledo District during 1940, it was numerically dominant inside houses during our survey. This species seemed undeterred by DDT residues because large numbers of *An. vestitipennis* females were captured inside both DDT-sprayed and unsprayed houses. Another intriguing aspect of this species' behavior relates to our capturing many more inside houses than were captured outdoors. The openness of many native houses in rural southern Belize probably facilitates this indoor-feeding behavior. In contrast, the host-seeking females of *An. apicimacula*, like *An. albimanus*, were much more abundant outdoors. Exophagic behavior of the latter 2 species should serve to diminish their overall vectorial capacity.

Recent studies by Loyola et al. (1991) and Padilla et al. (1992) have incriminated *An. vestitipennis* as a vector of human malaria in the Marqués de Comillas region of southern Mexico and in 2 communities in northern Guatemala, respectively. The latter studies, in combination with recent data compiled by Padilla in Guatemala (personal communication), show *An. vestitipennis* to be endophagic, but not as strongly endophagic as indicated by our data from Toledo District. Consequently, a greater sampling effort covering the entire nighttime interval will possibly show a greater relative tendency of *An. vestitipennis* females to feed outdoors in Toledo District.

The presence and abundance of malaria vectors are under the control of dynamic environmental variables, as well as human interventions. This report emphasizes the need to continuously study the changing roles of malaria vectors in different geographical areas. Based on the published literature, we can expect *An. darlingi*-transmitted malaria to respond favorably to a DDT house-spray program (Rozendaal et al. 1989, Roberts and Alecrim 1991). However, these expectations must be reevaluated if *An. vestitipennis* has become the primary vector of malaria in nonriverine areas of Toledo District.

Hopefully, this report will be the precursor of more definitive studies on vector responses to DDT-sprayed houses and on vectorial roles in different ecological zones of Belize.

We thank the staff of the Belize/United States Epidemiological Research Center for support and assistance. In particular we want to thank Robert Miller and Shilpa Hakre for their direct assistance. We thank Ralph Harbach for reviewing the manuscript. Special thanks are due Larry Barber and his staff at the Voice of America installation in Punta Gorda, Belize, for providing laboratory space and continuous assistance during our field work in the Punta Gorda area.

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Donald R Roberts
301-295-3734

VARIATION IN THE HINDTARSAL MARKINGS OF *ANOPHELES DARLINGI* (DIPTERA: CULICIDAE) IN BELIZE

RALPH E. HARBACH[1], DONALD R. ROBERTS[2] AND SYLVIE MANGUIN[2]

ABSTRACT. Aberrant phenotypes of *Anopheles darlingi* with basal dark scaling on either one or both of hindtarsomeres 3 and 4 are reported from Belize. Based on wild-caught females and adults reared from wild-caught larvae, it appears that approximately 8% of the natural population bears some degree of basal dark scaling on these hindtarsomeres. The occurrence of similar variants in other species of the subgenus *Nyssorhynchus* is summarized, and their significance in terms of inaccurate species identification is noted.

INTRODUCTION

Anopheles (*Nyssorhynchus*) *darlingi* Root has not been reported or collected from Belize since Komp (1940, 1941), Kumm and Ram (1941), and Walker (unpublished[3]). In fact, we thought this species had disappeared from Belize, perhaps due to agricultural and control practices, because we did not encounter it during intensive larval surveys conducted throughout the country in September 1990, April 1991, and September 1992 (Rejmankova et al. 1993, Roberts et al. 1993). In May 1993, however, we repeatedly collected adult females of this species from human bait both inside and outside houses located near rivers between Middlesex in Stann Creek District and Belmopan in Cayo District. Larvae were subsequently collected from riverine habitats near St. Thomas and Belmopan. *Anopheles darlingi* undoubtedly contributes in a major way to the increasing numbers of malaria cases in Belize (PAHO 1992).

Faran and Linthicum (1981) and Linthicum (1988) used the absence of dark scaling on hindtarsomeres 3 and 4 as the primary character for distinguishing adults of the subgenus *Nyssorhynchus* from those belonging to the other subgenera of *Anopheles* in the Neotropical Region. They indicated that hindtarsomeres 3 and 4 are entirely white in this group except in "unusual variants," which apparently refers to the so-called "mutants" and "anomalous specimens" of *An. albimanus* Wiedemann, *An. aquasalis* Curry, *An. strodei* (Root), and *An. triannulatus* (Neiva and Pinto) discussed by Faran (1980). Linthicum (1988) specifically listed *An. rondoni* (Neiva and Pinto) and *An. nigritarsis* (Chagas) as exceptions, but these species differ from the others in having basal dark bands constantly present on hindtarsomere 3 (*An. rondoni*) or both hindtarsomeres 3 and 4 (*An. nigritarsis*).

Faran (1980) divided the subgenus *Nyssorhynchus* into two sections, the Albimanus and Argyritarsis sections, but excluded four poorly known species of the "*Myzorhynchella* group," which was raised only recently to sectional status (Peyton et al. 1992). He distinguished adults of the Argyritarsis Section from those of the Albimanus Section primarily by the absence of a dark basal band on hindtarsomere 5. *Anopheles darlingi* is a member of the Argyritarsis Section, which is characterized by having hindtarsomeres 3–5

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[3] Four females of *An. darlingi* from Sierra de Agua (17° 30′ N 88° 50′ W), Orange Walk District, collected by A.J. Walker in June 1946 are deposited in the National Museum of Natural History, Smithsonian Institution. These specimens were examined and listed by Linthicum (1988).

entirely white-scaled (Faran 1980, Linthicum 1988). Of the eight species belonging to the Argyritarsis Section, dark markings on hindtarsomeres 3 and 4 have been observed only in *An. darlingi*. Most of the species mentioned above belong to the Albimanus Section. *Anopheles nigritarsis* belongs to the Myzorhynchella Section.

Komp (1942:37) mentioned that he had "specimens of *darlingi* from British Honduras [= Belize] with additional black hind tarsal bands," but this observation apparently went unnoticed by later authors because Komp never included it in a species description. We first noticed the presence of basal dark scaling on hindtarsomeres 3 and 4 in a few specimens of *An. darlingi* while identifying mosquitoes collected during malaria vector ecology studies involving the use of remote sensing. These mosquitoes were all frozen for *Plasmodium* detection and identification. The purpose of this paper is to bring attention to the presence of basal dark markings observed in adults of *An. darlingi* reared from wild-caught larvae and the progeny of wild-caught females that were frozen for isozyme analysis. There are no published reports of aberrant hindtarsal markings in populations of *An. darlingi* from any other country in Central or South America.

MATERIALS AND METHODS

Hindtarsal markings were examined in adults reared from wild-caught larvae and progeny broods obtained from females captured on human bait on May 25, 1993. Larvae were collected in shaded masses of floating plant debris and patches of *Cabomba* sp. along the edges of the Sibun River near St. Thomas (17° 09′ N 88° 37′ W) and Roaring Creek near Belmopan (17° 15′ N 88° 48′ W). The specimens were transported the next day to the Smithsonian Institution Museum Support Center in Suitland, MD. Larval collections and progeny broods were reared separately in plastic pans in an air-conditioned room held at 21 ± 1°C. Each pan was provided with straw for floatage and gently aerated (through a sandstone) by means of an aquarium pump. Fourth-instar larvae and pupae were removed from the pans and reared individually in plastic vials. Most of the adults reared from wild-caught larvae and a portion of those reared from each progeny brood were mounted on points on pins and examined for hindtarsal markings. All of these ~~experiments~~, along with their associated larvae and/or pupal exuviae, were deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC, for future study and reference. A number of adults (12%) obtained from progeny broods died upon emergence and could not be saved as voucher specimens. These specimens were examined for hindtarsal markings, then discarded along with their associated larval and pupal exuviae. The remainder of the adults (24% from wild-caught larvae and 39% from progeny broods) were examined while alive and subsequently frozen at −70°C for later biochemical studies. We retained larval and pupal exuviae from the frozen adults reared from wild-caught larvae. Two or three first-, second-, third-, and fourth-instar larvae from each progeny brood were preserved in 80% ethanol for future morphological study.

OBSERVATIONS

The variation observed in the hindtarsal markings of *An. darlingi* from Belize is illustrated in Fig. 1. Figure 1A shows the normal condition where the distal portion of hindtarsomere 2 and all of hindtarsomeres 3–5 are white-scaled. The other drawings in Fig. 1 illustrate variation in basal dark scaling sometimes present on the third and fourth hindtarsomeres: (1) dark scaling at the base of hindtarsomere 3 (Fig. 1B), (2) dark scaling at the base of hindtarsomere 4 (Fig. 1C), and (3) dark scaling at the bases of both hindtarsomeres 3 and 4 (Fig. 1D,E). The amount of dark scaling on hindtarsomeres 3 and 4 is variable, ranging from a narrow or incomplete ring to a broad, distinct band. The dark scaling, when present, usually forms complete bands. In general, when dark scaling is well developed on hindtarsomere 3, there is a corresponding reduction in the amount of

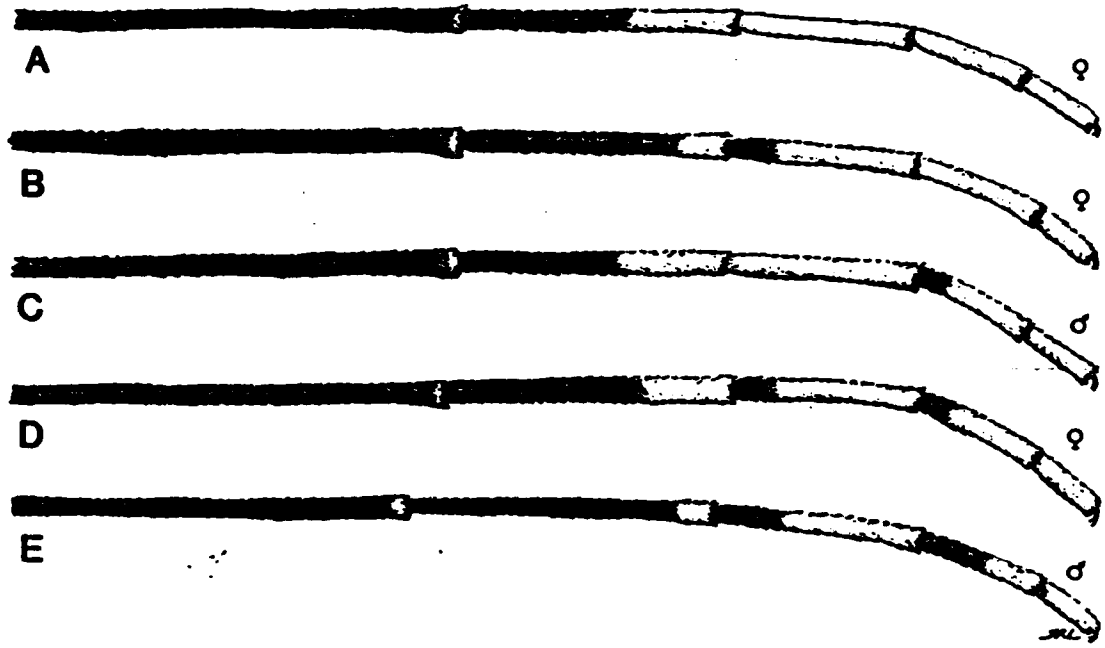

Fig. 1. Hindtarsal markings observed in specimens of *Anopheles darlingi* from Belize. A, Prevalent form, hindtarsomeres 3–5 entirely white-scaled; B–E, aberrant forms showing variation in basal dark scaling that sometimes occurs on one or both of hindtarsomeres 3 and 4.

white scaling on the distal portion of hindtarsomere 2 (Fig. 1B,E). In such cases, the extent of basal dark scaling on hindtarsomere 2 exceeds the range of "basal 0.35–0.55 dark" given by Linthicum (1988).

Ninety-four adults were reared from wild-caught larvae. Seven of these had basal dark scaling on one or both of hindtarsomeres 3 and 4. Six specimens (4♂, 2♀) exhibited the condition shown in Fig. 1C, i.e., basal dark scaling on hindtarsomere 4, and one female had basal dark scaling on both hindtarsomeres 3 and 4. If this sample is representative of the natural population of *An. darlingi* in Belize, then 7.4% (7/94) of the wild population would be expected to have dark markings on one or both hindtarsomeres 3 and 4. Of the seven individuals, 85.7% (6/7) had dark scaling at the base of hindtarsomere 4, and 14.3% (1/7) had dark scaling on both hindtarsomeres 3 and 4. Of the overall sample, 6.4% (6/94) had dark scaling at the base of hindtarsomere 4, and 1.1% (1/94) had dark scaling on the bases of both hindtarsomeres 3 and 4.

Progeny broods were obtained from 11 females. One female had a prominent dark band at the base of hindtarsomere 3, another had prominent dark basal bands on both hindtarsomeres 3 and 4, and the remainder (9) had normal hindtarsi. If these wild-caught females are grouped with the 94 adults reared from wild-caught larvae, then a total of 8.6% (9/105) of the natural population of *An. darlingi* in Belize would be expected to have some degree of dark scaling on hindtarsomeres 3 and 4. Two females among these individuals (1.9% of 105) had dark scaling on the bases of both hindtarsomeres 3 and 4, one female (1.0%) had dark scaling only at the base of hindtarsomere 3, and six individuals (4♂, 2♀) (5.7%) had dark scaling only on the base of hindtarsomere 4.

Of the progeny obtained from the female with dark scaling at the base of hindtarsomere 3, only nine survived to adulthood. One female was exactly like the mother in having dark scaling at the base of hindtarsomere 3, and one male differed in having dark scaling at the bases of both hindtarsomeres 3 and 4.

Three males and four females exhibited the normal condition shown in Fig. 1A. A total of 22.2% (2/9) of the individuals that survived from this brood had dark bands on the hindtarsomeres.

Only three offspring (2♂, 1♀) of the female with prominent dark bands at the bases of hindtarsomeres 3 and 4 survived to adulthood. Unfortunately, the single female emerged from the pupal exuviae without hindtarsi. These were left within the exuviae and could not be examined. The two males, however, had basal dark bands on hindtarsomeres 3 and 4 exactly like their mother. It is possible that this brood may have bred true, i.e., all of the progeny may have exhibited the maternal phenotype.

Of the progeny obtained from the other nine females, 119 (57♂, 62♀) survived to adulthood, and all but one of these were normal with respect to hindtarsomeres 3 and 4. A single female from a brood of 12 surviving adults (6♂, 6♀) had dark markings on the bases of hindtarsomeres 3 and 4. Therefore, 8.3% (1/12) of this brood exhibited dark hindtarsal markings. This is nearly the same ratio seen in wild-caught specimens.

DISCUSSION

Accurate identification of mosquitoes depends on a thorough knowledge of morphological variation within species. Variation in ornamentation sometimes leads to erroneous identification. In fact, lack of knowledge of variation in hindtarsal markings has led to the naming of a number of varieties and species that are conspecific, e.g., the *bisignatus* and *trisignatus* varieties of *An. albimanus* (Hoffmann 1938), the *guarauno* and *delta* varieties of *An. aquasalis* (Anduze 1948), *An. deltaorinoquensis* Cova Garcia, Pulido F. and Amanista M., which is a synonym of *An. aquasali* (Faran 1980), and *Cellia cuyabensis* (Neiva and Pinto), which is a synonym of *An. triannulatus* (Pinto 1939). Because morphological keys are designed by trained taxonomists primarily for the use of non-taxonomists and inexperienced identifiers, it is important that keys account for intraspecific variation as well as interspecific differences. In most published keys, specimens of *An. darlingi* with dark markings on hindtarsomeres 3 and 4 will key properly to the correct subgenus and species, but this is not so with the keys of Faran and Linthicum (1981) and Linthicum (1988), where reliance on the first or primary key character would cause these specimens to be misidentified at the subgeneric level. Fortunately, *An. darlingi* is a very distinct species in Belize, where it usually should be recognizable in both the adult and larval stages.

Among the subgenera of *Anopheles* in the Neotropical Region, hindtarsomeres 3 and 4 are not entirely white in *Anopheles, Lophopodomyia, Kerteszia,* and *Stethomyia.* As indicated above, these hindtarsomeres are entirely white in species of the *Nyssorhynchus* except for *An. nigritarsis* (Myzorhynchella Section), *An. rondoni* (Albimanus Section), and "unusual variants" (Faran and Linthicum 1981, Linthicum 1988). *Anopheles nigritarsis* is characterized by the constant presence of basal dark bands on hindtarsomeres 3 and 4 and *An. rondoni* by the constant presence of a dark basal band on hindtarsomere 3. Aberrant dark bands sometimes occur variously on one or more of hindtarsomeres 3–5 in certain populations of *An. albimanus, An. aquasalis, An. strodei,* and *An. triannulatus* of the Albimanus Section and *An. darlingi* of the Argyritarsis Section. A summary of additional dark bands observed in these species is given in Table 1. These are the variants that are likely to cause problems for non-taxonomists, particularly those who place too much emphasis on primary key characters, use them out of habit, or use them because they are more discrete and easier to observe than secondary key characters.

The phenotypes with aberrant hindtarsal bands observed in species of *Nyssorhynchus* have been called "mutants" and "anomalous specimens" by various authors (e.g., Rozeboom 1963, Kitzmiller and Mason 1967, Faran 1980). Indications are that these phenotypes are fairly common, especially in *An. albimanus, An. triannulatus,* and *An. darlingi.* From this study, it appears that approx-

Table 1. Summary of aberrant hindtarsal markings observed in species of the subgenus *Nyssorhynchus* of *Anopheles*.

Section	Species	Variants	Populations from	Principal references
Albimanus	*albimanus*	Basal dark bands on: 1) hindtarsomere 3 2) hindtarsomeres 3,4	Costa Rica, El Salvador, Guatemala, Texas (U.S.A.)	Hoffmann 1938, Rozeboom 1963, Faran 1980
	aquasalis	Apical dark bands on: 1) hindtarsomere 4 2) hindtarsomeres 3,4 Basal dark bands on: 3) hindtarsomeres 3,4	Venezuela	Anduze 1948, Faran 1980
	strodei	Basal dark bands on: 1) hindtarsomeres 3,4 2) hindtarsomere 4 3) hindtarsomeres 3,4 but absent on 5[1]	Brazil	Rachou and Ferraz 1951
	triannulatus	Basal dark bands on: 1) hindtarsomere 4 2) hindtarsomeres 3,4	Brazil	Pinto 1932, Galvão and Lane 1941
Argyritarsis	*darlingi*	Basal dark bands on: 1) hindtarsomere 3 2) hindtarsomere 4 3) hindtarsomeres 3,4	Belize	present study

[1] Species of the Albimanus Section are characterized by the presence of a basal dark band on hindtarsomere 5.

imately 8% of the *An. darlingi* in Belize have basal dark scaling on either one or both of hindtarsomeres 3 and 4, and the data suggest that a heritable genetic basis exists for the expression of this trait. Consequently, we prefer to characterize these phenotypes as "aberrant forms" or "normal variants," which imply deviation from the usual or prevalent form rather than a rare individual or strain resulting from mutation.

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Environmental and Regional Determinants of *Anopheles* (Diptera: Culicidae) Larval Distribution in Belize, Central America

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ABSTRACT Surveys of *Anopheles* larval habitats in northern Belize were carried out during September 1990 and April 1991. At each site, larvae were collected and the physical and chemical characteristics of water and species composition of aquatic vegetation were measured or estimated. Data on presence or absence of four species, *Anopheles albimanus* Wiedemann, *A. crucians* Wiedemann, *A. pseudopunctipennis* Theobald, and *A. argyritarsis* Robineau-Desvoidy, were used for analysis of associations with environmental factors, habitat types, and regions. Using significantly contributing environmental variables, discriminant functions (DF) were constructed for the *Anopheles* species, except for *A. argyritarsis* whose distribution could be predicted solely by altitude. The stability of DFs was checked by cross-validation runs. The DF for *A. pseudopunctipennis* was 93% accurate in predicting positive habitats. Predictions based on DFs for *A. albimanus* and *A. crucians* were 74 and 80% accurate, respectively. Of the four *Anopheles* species present in the study area, *A. albimanus* was the most common. Together with *A. crucians*, it occurred mostly on the coastal plain, and both species were present in both wet and dry seasons. *Anopheles albimanus* was positively associated with cyanobacterial mats and submersed–periphyton habitat types and negatively associated with the filamentous algae habitat type. *A. crucians* was positively associated with *Eleocharis*–periphyton habitat type. *A. pseudopunctipennis* and *A. argyritarsis* were common only during the dry season and their distribution was limited to the Karst and Mountain Pine Ridge regions. Both species were positively associated with the filamentous algae habitat type, and *A. argyritarsis* was also positively associated with the rock pools habitat type. Physical factors (e.g., water depth, water temperature, and oxygen content) were usually marginally correlated with larval occurrence. Dominant plant growth forms, such as filamentous algae, cyanobacterial mats, and submersed macrophytes showed the closest association with the larvae of particular *Anopheles* species. Our results demonstrated the controlling influence of dominant aquatic vegetation on larval presence.

KEY WORDS larval habitats, aquatic vegetation, *Anopheles* spp.

GEOMORPHOLOGY affects the hydrology of a region; i.e., distribution and seasonal dynamics of lakes, rivers, streams, and pools. Water quality in these different water bodies is influenced by rock and soil chemistry, vegetation of the surrounding landscape, and human activities. Both hydrology and water chemistry determine the type of aquatic vegetation present in lakes, pools, and streams. Shallow, quiet water with aquatic vegetation seems optimal for oviposition and larval development of most mosquito species. Descriptions of requirements of individual species for specific characteristics of larval habitats have generally been rather vague. A few attempts to describe the relationships between larvae and different environmental factors can be found in papers by Rioux et al. (1968), Hagstrum & Gunstream (1971), Hall (1972), Vrtiska & Pappas (1984), Gabinaud (1987), Orr & Resh (1989), Savage et al. (1990), and Rejmankova et al. (1991).

Obviously, if we can point out individual environmental factors related to the presence of larvae, then groups of individual factors are probably characteristic of specific larval habitats, which, in turn, might be related to distinct geo-

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graphic regions. Once the connections are made between the fine scale of individual larval habitats and a coarse scale of their regional distribution, our understanding of mosquito larval ecology, specifically with regard to malaria transmission, will be greatly improved.

The country of Belize, located south of the Yucatan Peninsula on the Atlantic coast of Central America (Fig. 1), provides a great variety of ecological settings as foci of malaria transmission. Komp (1941) first reported the occurrence of *Anopheles darlingi* Root in Belize. This finding was verified by Kumm & Ram (1941), who also documented the occurrence of malaria-infected specimens of *A. darlingi* and *A. vestitipennis* Dyar & Knab. Additionally, Kumm & Ram (1941) reported the presence of seven other species of *Anopheles;* i.e., *A. albimanus* Wiedemann, *A. pseudopunctipennis* Theobald, *A. punctimacula* Dyar & Knab, *A. apicimacula* Dyar & Knab, *A. eiseni* Coquillett, *A. argyritarsis* Robineau-Desvoidy, and *A. crucians* Wiedemann. Bertram (1971) reported collecting all of these species, except *A. darlingi,* in Belize. Bertram's work, which emphasized the ecology of adult mosquitoes, is practically the only source of information on the spatial and seasonal distribution of anophelines in Belize.

Not only in Belize but throughout Central America, larval ecology of malaria vectors has been the subject of infrequent and sporadic studies. Review papers by Rozeboom (1941), Watson & Hewitt (1941), and Bates (1949) described the seasonal and spatial distribution of *A. albimanus* and *A. pseudopunctipennis.* Breeland (1972) presented specific information on the seasonal and spatial distribution of these vectors along the Pacific coast of El Salvador. Bailey et al. (1981) studied the distribution of *A. albimanus* larvae in estuarine habitats of El Salvador. The relationships of *A. albimanus* and *A. pseudopunctipennis* larvae to dominant aquatic plants and environmental factors in southern Chiapas, Mexico, have been reported by Savage et al. (1990) and Rejmankova et al. (1991). A hierarchical method for classifying larval habitats into habitat types was subsequently suggested by Rejmankova et al. (1992).

In addition to *A. albimanus* and *A. pseudopunctipennis,* several other *Anopheles* occur in Central America. Recently, *A. vestitipennis,* previously considered to be a relatively unimportant malaria vector, was found to transmit malaria in areas within Mexico and Guatemala (Loyola et al. 1991, Padilla et al. 1992). Roberts et al. (1993) found this species to be of potential importance as a vector of malaria in Belize. These recent findings are indicators of our poor understanding of vectorial roles of *Anopheles* in much of Central America. Malaria rates in Belize are increasing, so the issues of species distributions and vectorial roles are increasingly important to the health and welfare of the Belizean population.

An array of vegetation types exists in Belize. Most of the primary tropical deciduous forests have been disturbed by intensive logging for mahogany and logwood and traditional slash-and-burn agriculture. Extensive areas on the coastal plain are covered with seasonally inundated savanna, lowland pine forest, and freshwater swamp forest. Mangrove swamps are common along the coast and extend inland wherever brackish water occurs. Sugarcane, grown mostly in northern Belize, is a prime agricultural crop. Citrus-growing is becoming more important, with large areas of forest in the Cayo and Stann Creek districts currently being cleared for citrus cultivation.

In September 1990, we initiated a surveillance program to obtain population-based data on the malaria vectors in Belize. The quantity of environmental data compiled was greater than normally collected in field surveys. This allowed the larval–environmental associations to be studied from different levels of detail, ranging from the individual habitat to a regional level. The most detailed analysis was performed at the individual habitat level, using environmental variables that might affect oviposition as well as larval distribution, density, development, and survival. A second approach was based on a more holistic view of larval habitats. Using this approach, habitats were described according to their dominant vegetation, classified into habitat types, then examined for association between habitat types and the presence or absence of *Anopheles* species. The third approach to data analysis involved assessment of associations at the regional level.

Program objectives were to document which vector species were present in northern Belize, to define the habitat ranges of these species, and to determine whether their presence or absence could be predicted by environmental factors, habitat types, or regional characteristics. Reported herein are the results of habitat analysis and regional distribution of *A. albimanus, A. pseudopunctipennis, A. crucians,* and *A. argyritarsis.*

Materials and Methods

Study Area. With an area of 23,000 km^2 and a population of ≈180,000, Belize is a country with the lowest population density in Central America. Lowlands of Belize are characterized by a variety of wetlands, freshwater and brackish, seasonal and permanent. Montane and foothill regions include many streams and rivers. The hydrological and vegetational diversity results in a wide variety of mosquito larval habitats.

The amount of rainfall increases from ≈1,300 mm annually in the north to 2,400 mm around Belize City. The normal dry season is from January through April and is shorter and less severe

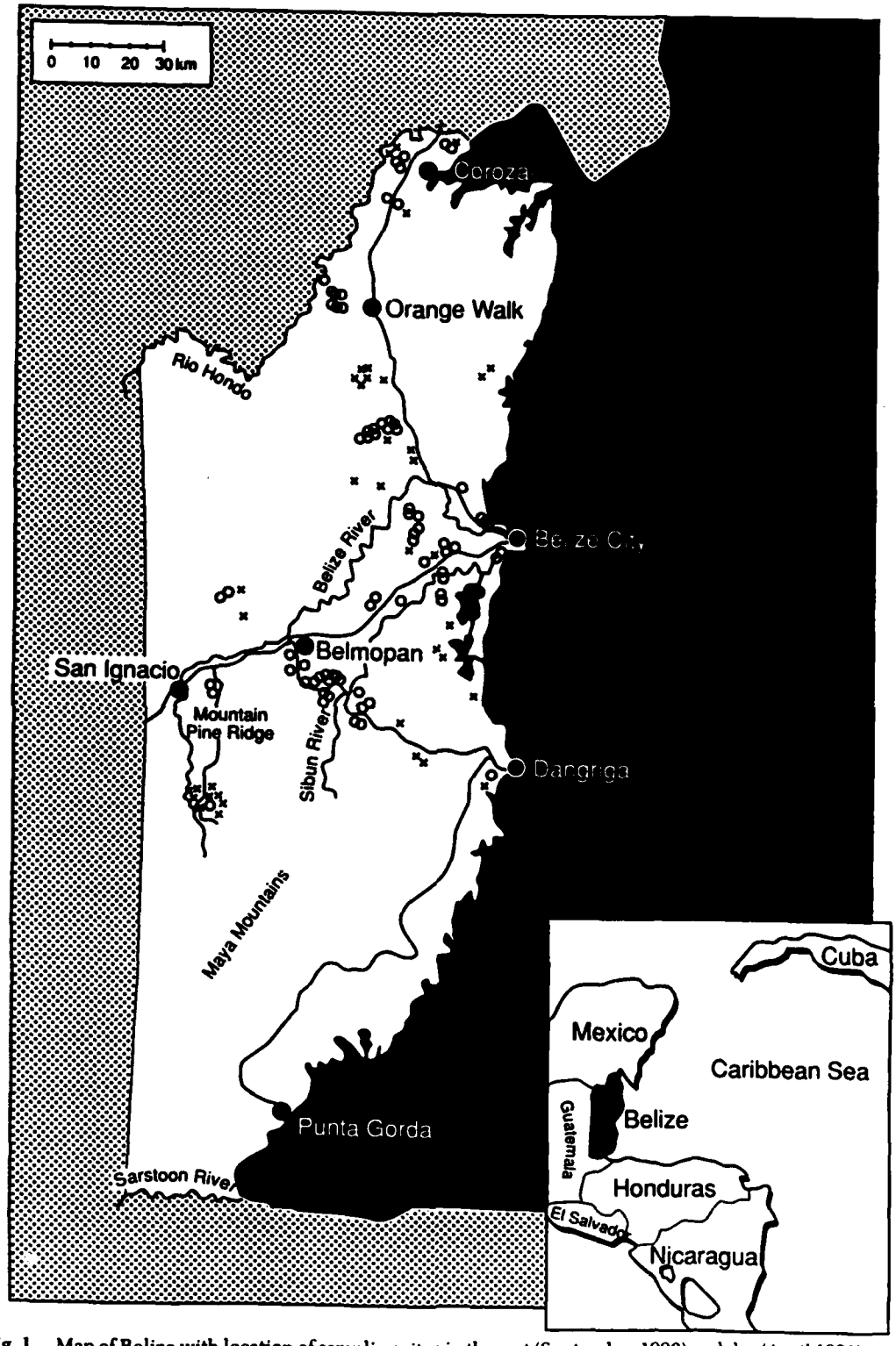

Fig. 1. Map of Belize with location of sampling sites in the wet (September 1990) and dry (April 1991) seasons. Circles indicate sites visited during both seasons. Crosses indicate sites added in the dry season.

than at comparable latitudes on the Pacific coast of Central America.

Our survey sites were distributed in the northern part of Belize from Dangriga north, covering Corozal, Belize, Orange Walk, and parts of Cayo and Stann Creek districts (Fig. 1). This northern area includes three distinct physiographic regions: flat coastal and inland plain (CP), karst and foothills (KARST), and Mountain Pine Ridge (MPR), which all differ in their topography, geology, hydrology, soils and, consequently, vegetation cover (Hartshorn et al. 1984). The terms "regional" and "region" are used in this article for physiographic regions of northern Belize on a scale of 10^2–10^3 km^2.

The MPR region includes fast-flowing rivers and streams with nutrient-poor waters of very low mineral content. No extensive wetlands occur in this region; therefore, mosquito habitats exist in the form of river pools with filamentous algae and occasional graminoids. In the KARST region, larval habitats are also mostly associated with rivers. These rivers are slower than in the MPR and their waters are richer in minerals, specifically calcium. Pasture ponds and small lagoons with different types of aquatic vegetation are also present in this region. The CP includes both fresh and brackish waters and provides very diverse and often extensive habitats ranging from almost monospecific marshes dominated by a sedge, *Eleocharis interstincta* (nomenclature for vascular plants follows Standley & Steyermark 1946–1977), to species-rich ponds and lagoons.

Larval Sampling. Surveys of a wide range of mosquito larval habitats were conducted in the northern part of Belize in both the wet (September 1990) and dry (April 1991) seasons. A mosquito larval habitat is defined as a body of water with uniform vegetation and a specific water chemistry (Rejmankova et al. 1992). In the wet season, larval habitats were sampled at 75 different sites (see Fig. 1). In the dry season, the 75 sites previously surveyed in the wet season were visited and some new sites were added because many of the wet-season locations were dry. The total number of sites with water in the dry season including both the old and added sites was 73.

The following data were recorded for each habitat: total percentage of emergent, floating, or submersed vegetation, algal mats, and detritus; percentage of cover of individual plant species; amount of phytoplankton (measured fluorometrically as chlorophyll *a* concentration); water conductivity; pH; and dissolved oxygen. Water analyses were conducted for total suspended solids, particulate organic matter, nitrate and ammonia nitrogen (NO_3, NH_4), orthophosphate phosphorus (PO_4), and major cations (Na, K, Ca, Mg) using standard limnological methods (APHA 1985). Thirty dips for mosquito larvae were taken from each habitat. Although a greater number of dips was not practically feasible, we already knew from earlier work that 30 dips provided a rough estimate of population density (Savage et al. 1990; Rejmankova et al. 1991). To process the samples, larvae and pupae were transported to the laboratory in Belize City and reared to obtain adults with associated immature exuviae for identification, study, and future reference. Some fourth instars were also preserved from most collections.

Larval Occurrence and Environmental Factors. Data on the occurrences of larvae of different *Anopheles* species were related to environmental factors. Because of large variations in larval density, most analyses were conducted using information on the presence–absence of individual species. The environmental variables were subjected to either log transformation (conductivity) or the angular transformation (all plant variables were expressed as percentage values) before further analysis. The two-tailed *t* test was used to compare the group means of environmental variables for sites with or without larvae.

Discriminant Analysis. Relationships between the presence–absence of each *Anopheles* species in the dry season data set and the selected environmental variables were further explored using discriminant analysis (Tabachnick & Fidell 1989). Our goal was to select a reduced set of variables for predicting the distribution of each species. The discriminant functions were first calculated using all the environmental variables identified by *t* test as having significantly different group means for sites with and without larvae. Subsequently, the variables that did not contribute significantly to the respective discriminant functions were deleted. The final number of variables used was four for *A. albimanus* and *A. crucians* and three for *A. pseudopunctipennis*. We did not calculate the discriminant function for *A. argyritarsis*, whose distribution could be predicted solely by altitude.

To assess the predictive power of the respective discriminant functions, five randomly selected subsets of data were used to calculate the functions that were subsequently applied to independent data subsets (cross-validation technique; see Tabachnick & Fidell 1989).

Habitat Types. Because of substantial habitat diversity, the individual habitats, defined by dominant plant species, were categorized into higher units, subsequently referred to as habitat types (Rejmankova et al. 1992). Cluster analysis (Orloci 1978) based on the absolute distance dissimilarity after the angular transformation of the environmental variables (plant cover) was used for delineation of nine habitat types based on the wet-season data. During dry-season sampling, a site was ascribed to a habitat type before sampling for larvae was done. Three additional distinctive habitat types were sampled in the dry season: rock pools without filamentous algae, detritus, and planktonic algae. Based on the aver-

Table 1. Average specific conductivity ± SD for sampling sites in the four regions in the wet and dry seasons

Season	Mountain pine ridge	Karst	Coastal plain	
			Fresh	Brackish
Wet	14	144 ± 129	91 ± 71	2,186 ± 820
Dry	42 ± 12	196 ± 216	183 ± 97	1,826 ± 891

age number of larvae per dip, the habitat types were ranked as high (>1), medium (0.1–1), and low (<0.1).

Larval Distribution Within Defined Habitat Types and Geographic Regions. *G* tests of independence (Zar 1984) were calculated to determine the associations between the presence–absence of each vector species and habitat types and regions, respectively.

Results

Dry-season sampling revealed that 66% of wet-season habitats were dry during the dry season, 7% were significantly smaller, and 27% were relatively unchanged. Water conductivity was significantly higher in the dry season in habitats in both MPR and CP (fresh), whereas it did not differ much in KARST and CP (brackish) (Table 1). Plant diversity was much higher in the wet season than in the dry season (see list of plant species in Appendix 1), mainly because species-rich edges of ponds and lagoons that were flooded during the wet season dried up and ceased being larval habitats during the dry season.

Larval Occurrence and Environmental Factors. Physical factors (e.g., water depth, water temperature, oxygen content) were usually marginally correlated with larval occurrence. Dominant plant growth forms such as filamentous algae, cyanobacterial mats, and submersed macrophytes showed the closest association with the larvae of particular *Anopheles* species.

Discriminant Analysis. Using the environmental variables with significantly different group means for sites with larvae present versus absent (Tables 2 and 3), we calculated discriminant functions for the dry season for all the *Anopheles* species (Fig. 2 a–c), except for *A. argyritarsis*.

For *A. albimanus*, 10 environmental variables were significantly different for dry-season sites with and without larvae (Table 2). Of these variables, only cover percentage of submersed plants, cover percentage of cyanobacterial mats, altitude, and temperature contributed significantly to the discriminant function by 44, 30, 14, and 12%, respectively. The discriminant function for the whole data set correctly predicted the presence of larvae in 74% of all sites and correctly predicted the absence of larvae in 91% of the sites (Fig. 2a; Table 4). Five randomly selected subsets of data were then used to construct the discriminant functions. When these functions were tested on the remaining independent subsets of data, the correctly predicted percentage of sites with larvae varied from 45 to 89%. Additionally, 81–95% of sites without larvae were correctly classified (Table 4). Cover percentage of periphyton, detritus, and emergent plants and habitat area were used as variables for constructing discriminant functions for *A. crucians* (Fig. 2b; Table 5) contributing by 44, 24, 22, and 10%, respectively, to the predictive power of the DF. Using the entire data set, the function correctly classified 80% of sites for the presence of larvae and 94% for the absence of larvae. Using five randomly selected subsets, correct predictions varied from 33 to 100% and from 84 to 91% for presence and absence of larvae, respectively. Cover percentage of filamentous algae, altitude, and water depth were used to construct the discriminant function for *A. pseudopunctipennis* (Fig. 2c; Table 6) contributing 81, 11, and 8%, respectively, to the predictive power of the DF. With the entire data set, the function correctly classified 93% of the sites for presence of larvae and 93% for absence of larvae. For the five randomly selected subsets, correct predictions ranged from 78 to 100% for positive sites and from 87 to 100% for negative sites.

Habitat Types. During the wet and dry season collections, nine and 12 major habitat-types were distinguished respectively, as defined by a dominant plant species, genera or life form (Fig. 3; Table 7). Of these twelve habitat types, five represented emergent macrophytes (including mangroves), two belonged to floating hydrophytes, and three were characterized by submersed hydrophytes. Three remaining habitat types (rock pools with no filamentous algae, detritus, and planktonic algae) did not contain any macrophytic vegetation. The detailed description of habitat types is given in Appendix 2. As shown in Table 7, habitat types cyanobacterial mats, submersed macrophytes–periphyton, *Nymphaea–Limnanthemum*, and mangroves were relatively stable with three of five, two of five, three of seven, and two of five sites staying the same in both the wet and dry seasons, respectively. Most sites sampled during the wet season that belonged to graminoids and *Eleocharis interstincta*–periphyton habitat types and all sites of *Typha–Cladium* habitat type were dry during the dry season. Three sites of wet-season graminoids habitat type, two sites of cyanobacterial mats habitat type, and one site each of *Eleocharis interstincta*–periphyton and *Nymphaea–Limnanthemum* habitat types developed into different habitat types during the transition from wet to dry seasons.

Larval Distribution Among Habitat Types. The tendency of water bodies to contain the same habitat type in both seasons (16 of 25) and, consequently, to support larvae of the same spe-

Table 2. Comparison of significantly different group means (±SD) of environmental variables measured in the dry season (two-tailed *t* test)

Environmental variable	Larvae		$P <$ *
	Present	Absent	
	Anopheles albimanus		
No. sites	27	46	—
% Cyanobacterial mats	20.4 (31.5)	1.8 (10.3)	0.0001*
% Submersed	29.0 (38.1)	2.1 (11.7)	0.0001*
% Filamentous algae	3.3 (17.3)	17.4 (28.0)	0.005*
Habitat area, m^2	25.6 (41.3)	7.5 (11.2)	0.006
Water body area, m^2	1,848.8 (2,600.0)	644.8 (1,200.0)	0.009
% Periphyton	5.8 (16.1)	0.6 (1.5)	0.01*
Altitude, m	35.3 (56.9)	118.3 (171.8)	0.02
Conductivity μmhos/m	1,086.5 (1,087.0)	738.9 (1,610.6)	0.03+
Temperature, °C	32.8 (2.1)	31.6 (1.9)	0.03
Oxygen, ppm	8.6 (3.7)	7.1 (2.6)	0.058
	Anopheles crucians		
No. sites	10	63	—
% Periphyton	12.9 (25.3)	0.9 (2.2)	0.0001*
% Emersed	28.2 (28.9)	11.4 (23.0)	0.01*
% Detritus	12.0 (28.2)	1.9 (9.7)	0.02*
Habitat area, m^2	30.0 (60.2)	11.6 (18.0)	0.05
	Anopheles pseudopunctipennis		
No. sites	14	59	—
% Filamentous algae	48.9 (31.1)	3.5 (13.5)	0.0001*
Conductivity, μmhos/m	102.0 (94.6)	1,049.1 (1,549.5)	0.0002+
Water depth, cm	8.0 (9.45)	24.0 (15.5)	0.0004
Altitude, m	192.1 (178.0)	62.7 (126.4)	0.002
% Emersed	0.07 (0.26)	17.0 (26.1)	0.004*
Temperature, °C	30.9 (2.5)	32.3 (1.9)	0.02
Water body area, m^2	1.8 (2.6)	1,348.0 (2,054.0)	0.02
% Submersed	0.0 (0.0)	14.9 (30.4)	0.03*
Habitat area, m^2	1.4 (1.5)	17.1 (30.1)	0.05
	Anopheles argyritarsis		
No. sites	9	64	—
Altitude, m	453.0 (40.0)	36.0 (47.5)	0.0001
Conductivity, μmhos/m	42.2 (12.7)	983.5 (1,504.2)	0.0001+
Temperature, °C	33.9 (1.6)	31.8 (2.0)	0.005
% Emersed	0.11 (0.33)	15.6 (25.5)	0.03*
Water body area, m^2	0.8 (0.8)	1,243.3 (2,004.6)	0.058

* *, after angular transformation; +, after log transformation.

Table 3. Comparison of significantly different group means (±SD) of environmental variables measured in the wet season (two-tailed *t* test)

Environmental variable[a]	Larvae		$P <$ [b]
	Present	Absent	
	Anopheles albimanus		
No. sites	18	57	—
% Cyanobacterial mats	24.4 (24.5)	3.51 (7.26)	0.0001*
POM, ppm	53.26 (94.84)	3.55 (4.19)	0.0001
Ca^{++}, ppm	168.41 (206.26)	52.67 (80.75)	0.0009
TSS, ppm	69.92 (121.27)	9.77 (11.16)	0.0004
Mg^{++}, ppm	54.65 (67.25)	16.91 (31.85)	0.002
% Detritus	8.16 (16.05)	1.28 (4.34)	0.003*
pH	7.57 (0.63)	7.10 (0.80)	0.03
	Anopheles crucians		
No. sites	9	66	—
pH	6.63 (0.76)	7.30 (0.76)	0.02

[a] POM, particulate organic matter; TSS, total suspended solids.
[b] *, after angular transformation; +, after log transformation.

cies, was significant (*G* test; $P < 0.025$). Larval density was much higher in the dry season than in the wet season (Fig. 3). All four *Anopheles* species were present in the dry season, whereas only *A. albimanus* and *A. crucians* were found in the wet season. In the dry season, cyanobacterial mats, filamentous algae, and submersed–periphyton represented high larval density habitat types (>1 larva per dip); *Eleocharis*–periphyton, broadleaved, rock pools, detritus, and planktonic algae belonged to medium-density habitat types (0.1–1 larva per dip); and the rest were low-density habitat types (<0.1 larva per dip). In the wet season, high densities of larvae were found in cyanobacterial mats and filamentous algae habitat types, the graminoids habitat type produced medium numbers of larvae, and the remaining habitat types produced very few larvae. Because of a large variability in larval counts and a low number of replicates, we did not find statistically significant differences in larval density between habitat types (Sheffe multiple comparison test), except for a wet-season difference between cyanobacterial mats and all remaining habitat-types.

The results of the *G* test of independence between habitat types and *Anopheles* species (Fig. 4) show a highly significant positive association between *A. albimanus* and the cyanobacterial mats and submersed–periphyton habitat types, and a highly significant negative association between *A. albimanus* and filamentous algae habitat type. *A. crucians* was positively associated with the *Eleocharis*–periphyton habitat type and slightly negatively associated with the filamentous algae habitat type. *A. pseudopunctipennis* and *A. argyritarsis* were positively associated with the filamentous algae habitat type, and *A. argyritarsis* was positively associated with the rock pools (no algae) habitat type.

Regional Distribution. Fig. 5 summarizes the data on distribution of *Anopheles* species among the different regions of the study area. *A. argyritarsis* was found only in rock pools of MPR. The rock pools are characterized by low water conductivity with very low content of minerals. *A. pseudopunctipennis* occurred in both MPR and KARST, always in river pools with filamentous algae. Water in the KARST region has a higher content of minerals, specifically calcium (>20 ppm). *A. crucians* was found mainly in habitats associated with CP (fresh) (water conductivity comparable to KARST), even though it was occasionally present in KARST and CP (brackish) as well. The highest larval densities of *A. albimanus* were found in habitats of CP (brackish), but this species was quite common in KARST and CP (fresh) as well. Statistical significance of these associations is expressed in Fig. 6.

Discussion

Discriminant Functions. The discriminant functions for presence of *A. albimanus* and *A. pseudopunctipennis* using data from southern Chiapas, Mexico, were published by Savage et al. (1990). The authors used slightly different techniques to construct their DF and select the significant variables. Yet the final selection of important variables for *A. pseudopunctipennis* was the same as in this paper; i.e., filamentous algae, altitude, and water depth. Consequently,

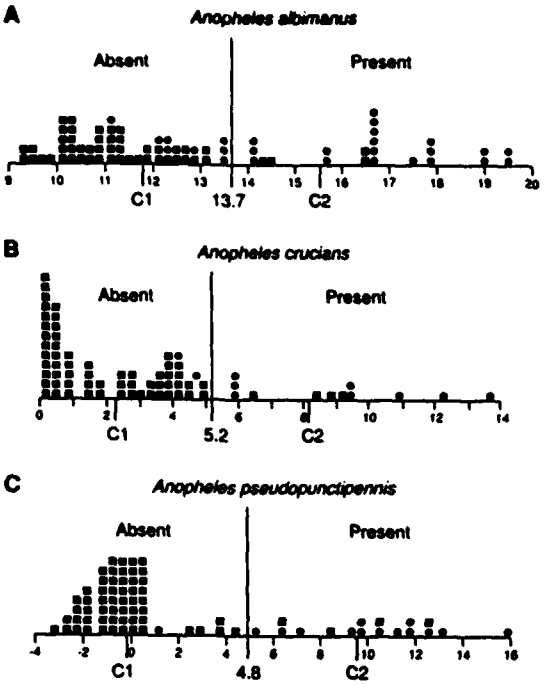

Fig. 2. (A) Discriminant function (Z, see Table 4) for *A. albimanus*. ■, Species absent; ○, species present. C1 and C2, Group centroids. (B) Discriminant function (Z, see Table 5) for *A. crucians*. ■, Species absent; ○, species present. C1 and C2, Group centroids. (C) Discriminant function (Z, see Table 6) for *A. pseudopunctipennis*. ■, Species absent; ○, species present. C1 and C2, Group centroids.

we are quite confident that the DF for *A. pseudopunctipennis* is broadly applicable to other northern areas of Central America. The environmental variables used, especially the cover percentage of filamentous algae that contributes ≈80% to the predictive power of the DF, appear to exert a controlling influence on the distribution of this species.

Predictions based on the DF for *A. albimanus* were less accurate than those for *A. pseudopunctipennis*. The DF for *A. albimanus* could not be compared with that of Savage et al. (1990) because their function included the cover of *Eichhornia*, a floating aquatic macrophyte, as one variable; *Eichhornia* was not found in Belize. The variables selected for DF were, in descending order of importance, submersed macrophytes, cyanobacterial mats, altitude, and water temperature. When the DF derived from the dry-season data was applied to the wet-season data set, it resulted in 72% of correctly predicted positive sites and 49% of correctly predicted sites with larvae absent. This is (at least for the positive sites) in the range of predictive values found for *A. albimanus*. The lower predictive value of *A. albimanus* DFs compared with DFs for *A. pseudopunctipennis* may be caused by the broader range of environmental conditions under which *A. albimanus* larvae occur. Variables associated with the presence of *A. albimanus* larvae in habitats in Belize were quite different from those in Mexico. In Mexico, the main variables were phytoplankton (unicellular green algae) in both seasons and *Eichhornia* in the dry season and Cyperaceae and phosphates in the wet season. None of these variables was linked with the distribution of *A. albimanus* larvae in Belize. Few habitats supported measurable quantities of phytoplankton in Belize, whereas many habitats were rich in phytoplankton in Mexico. This may be because waters in southern Chiapas contained generally 2–3 times higher concentrations of major nutrients (nitrogen, phosphorus) because of the volcanic origin of nutrient-rich soils in the area, abundant cattle manure, and extensive use of fertilizers. In the limestone regions of Belize, waters were poor in nitrogen and phosphorus but rich in calcium, both conditions being rather unfavorable for the growth of phytoplankton. On the other hand, extensive benthic cyanobacterial mats capable of nitrogen fixation, and submersed macrophytes overgrown with periphyton, were quite common in Belize, but they were not encountered in habitats in Mexico. Stands of several Cyperaceae species

Table 4. Cross-validation of discriminant functions for *A. albimanus* using five randomly selected data subsets

Subset no.	Derived from P/A[a]	Applied to P/A[a]	% Correctly predicted		Coefficients				Cut-off value
			As present	As absent	d_1	d_2	d_3	d_4	
1	18/19	9/27	89	81	0.526	−0.008	3.789	2.578	17.57
2	16/21	11/25	45	92	0.946	−0.008	4.624	6.208	32.61
3	9/28	18/18	72	94	0.263	−0.005	5.904	7.262	10.68
4	13/24	14/22	64	91	0.297	−0.005	3.994	3.947	11.08
5	11/26	16/20	69	95	−0.121	−0.003	7.007	3.400	−2.24
The whole data set			74	91	0.387	−0.006	4.428	4.306	13.68

General form of equation: $Z = d_1(T) + d_2(Alt) + d_3\arcsin(SB)^{1/2} + d_4\arcsin(BG)^{1/2}$, where T, temperature (°C); Alt, altitude (m); SB, submersed macrophytes (cover percentage after angular transformation); BG, cyanobacterial mats (cover percentage after angular transformation).

[a] P, number of sites with species present; A, number of sites with species absent.

Table 5. Cross-validation of discriminant functions for *A. crucians* using five randomly selected data subsets

Subset no.	Derived from P/A[a]	Applied to P/A[a]	% Correctly predicted		Coefficients				Cut-off value
			As present	As absent	d_1	d_2	d_3	d_4	
1	6/31	4/32	50	84	0.028	5.409	24.154	8.004	5.80
2	6/31	4/32	50	91	0.043	3.663	15.128	18.039	6.92
3	6/31	4/32	75	91	0.006	4.846	11.347	4.852	3.74
4	5/32	5/31	100	91	0.014	3.111	5.527	7.613	2.93
5	4/33	6/30	33	93	0.002	4.207	13.738	21.631	6.24
The whole data set			80	94	0.034	4.785	13.429	9.712	5.20

General form of equation: $Z = d_1(HA) + d_2\arcsin(EM)^{1/2} + d_3\arcsin(PER)^{1/2} + d_4\arcsin(DET)^{1/2}$, where HA, habitat area (m^2); EM, emergent macrophytes (cover percentage after angular transformation); PER, periphyton (cover percentage after angular transformation); DET, detritus (cover percentage after angular transformation).

[a] A, number of sites with the species absent; P, number of sites with the species present.

were present in Belize, but they did not support comparably high densities of *A. albimanus* larvae as in Mexico.

The DF for *A. crucians* was about as accurate as the DF for *A. albimanus*. Similarities in predictive accuracy of DFs for the two species reflect the tolerance of both species to a wide variety of habitats.

The fourth *Anopheles* included in the analysis, *A. argyritarsis*, was strictly associated with higher altitudes. Although this species was collected only at higher elevations, other collection records reveal that populations of *A. argyritarsis* also occur at lower elevations in KARST (Bertram 1971; unpublished observation). Therefore, any final conclusions about the association of this species with higher altitudes must await additional data.

Habitat Types. A second approach to larval analysis was based on the classification of habitats into habitat types according to their dominant vegetation. With 12 habitat types derived from 73 sampling sites, there were not enough replicates of each habitat type for detailed statistical analysis. However, using a *G* test of independence, several significant associations were found between mosquito larvae and habitat types. We were also able to rank the habitat types into groups of high, medium, and low densities of larvae. In our previous article (Rejmankova et al. 1992), we pointed out that, in addition to knowing whether habitats are associated with low, medium, or high larval densities, we also need to know the spatial and temporal extent of habitats to estimate their contribution to mosquito production. For example, habitat types of cyanobacterial mats and submersed–periphyton are in the high larvae-producing group in the dry season, whereas only cyanobacterial mats continue as high producers during the wet season. Evaluating the spatial distribution of individual habitat types in the regions should be a next step in our research effort.

Regions. Certain habitat types are related to specific regions, and they reflect the regional geology, hydrology, water, and soil quality. The MPR provides only two habitat types related to fast-flowing rivers and streams; i.e., rock pools and filamentous algae. The filamentous algae habitat type was not found very frequently in this region, probably because of a very low nutrient content of water. It is highly probable, however, that if streams and rivers from MPR became polluted, they would support more vigorous growth of filamentous algae and would provide a suitable habitat for *A. pseudopunctipennis* larvae. KARST is more diverse than MPR, but the most common habitat type (particularly in the dry season) was filamentous algae with associated populations of *A. pseudopunctipennis*. Populations of *A. albimanus* and *A. crucians* were found rather infrequently in KARST. Diverse fresh and

Table 6. Cross-validation of discriminant functions for *A. pseudopunctipennis* using five randomly selected data subsets

Subset no.	Derived from P/A[a]	Applied to P/A[a]	% Correctly predicted		Coefficients			Cut-off value
			As present	As absent	d_1	d_2	d_3	
1	8/29	6/30	100	100	0.004	−0.025	5.61	2.21
2	6/31	8/28	86	87	0.004	−0.092	24.50	7.73
3	5/32	9/27	78	93	0.005	−0.005	14.29	6.44
4	9/28	5/31	80	94	0.009	−0.120	11.28	3.37
5	7/30	7/29	100	90	0.010	−0.017	8.82	4.33
The whole data set			93	93	0.008	−0.050	12.32	4.79

General form of equation: $Z = d_1(Alt) - d_2(WD) + d_3\arcsin(FA)^{1/2}$, where Alt, altitude (m); WD, water depth (cm); FA, filamentous algae (cover percentage after angular transformation).

[a] P, number of sites with species present; A, number of sites with species absent.

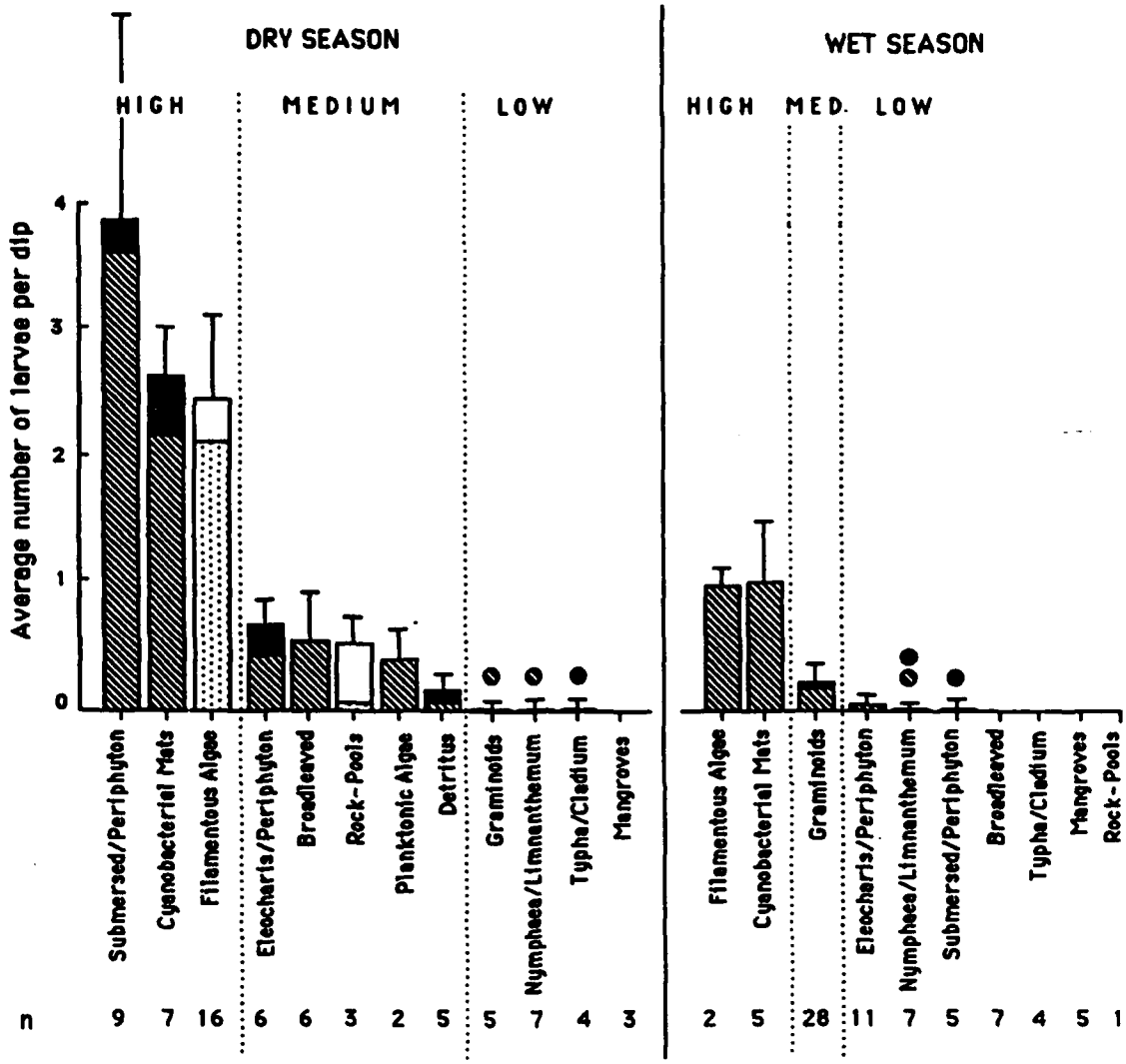

Fig. 3. Distribution of *Anopheles* species expressed as the average number of larvae per dip in individual habitat types. For the habitat description, see text. The number of sampling sites (*n*) belonging to each habitat type is indicated under the figure. Vertical bars indicate the standard error of mean. Wet season, September 1990; dry season, April 1991. For species description, see Fig. 5.

brackish water habitat types supporting both *A. albimanus* and *A. crucians* populations were encountered in CP. The habitat type cyanobacterial mats, which supports *A. albimanus*, was more frequent in CP (brackish). Habitat-types *Eleocharis*–periphyton and submersed–periphyton were common in CP (fresh).

During the wet season, neither *A. argyritarsis* nor *A. pseudopunctipennis* were found, most probably because their habitats were constantly flushed by heavy rains. This was similar to earlier findings in southern Mexico (Savage et al. 1990). Permanent bodies of water generally had the same habitat type and the same *Anopheles* species in both seasons. Larval densities were generally higher during the dry season than during the wet season; these differences may be related to smaller volumes of water being available in the dry season.

It is interesting that no environmental factors related to water chemistry, such as individual cation or anion concentrations, total suspended solids, or particulate organic matter, were found to be significantly correlated with the occurrence of larvae, except for *A. albimanus* in the wet season. Of all the environmental factors considered, dominant plant growth forms such as filamentous algae, cyanobacterial mats, submersed macrophytes, etc., showed the closest association with the larvae of particular *Anopheles* species.

Table 7. Transition of habitat-types from wet to dry season

Habitat type[a]	Sampled in wet season Total	Transition period from wet to dry season		Sampled in dry season		
		Dried	Contained water	Extant	Added	Total
BG	5	0	5	4	3	7
N–L	7	3	4	3	4	7
S–P	5	3	2	4	5	9
E–P	11	7	4	3	3	6
Br	7	5	2	0	6	6
Gr	28	23	5	4	1	5
T–C	4	4	0	2	2	4
FA	2	2	0	2	14	16
Ma	5	3	2	2	1	3
RP	1	0	1	1	2	3
De	0	0	0	0	5	5
PA	0	0	0	0	2	2

Numbers of habitats belonging to individual habitat types sampled in the wet season, dried out during the transition from the wet to dry season, containing water even in the dry season, added in the dry season, and total sampled in the dry season. Change from one habitat type to another during the transition period is indicated by arrows.

[a] BG, cyanobacterial mats; N–L, *Nymphaea–Limnanthemum*; S–P, submersed macrophytes–periphyton; E–P, *Eleocharis interstincta*–periphyton; Br, broadleaved; Gr, graminoids; T–C, *Typha–Cladium*; FA, filamentous algae; Ma, mangroves; RP, rock pools; De, detritus; PA, planktonic algae.

Physical factors (e.g., water depth, water temperature, and oxygen content) were usually marginally correlated with larval occurrence. This makes results of the analyses based on individual environmental factors very similar to those based on habitat types because the habitat types were defined by dominant plant forms.

The data presented here will eventually be used to develop a geographic information system on the distribution of malaria vectors in northern Belize. The analyses have led to additional questions related to malaria vector ecology: How soon do *A. argyritarsis* and *A. pseudopunctipennis* habitats develop in the dry season? How will the changes in land use (establishment of citrus plantations, increases in human population and migration, etc.) affect distribution and density of mosquito larval populations?

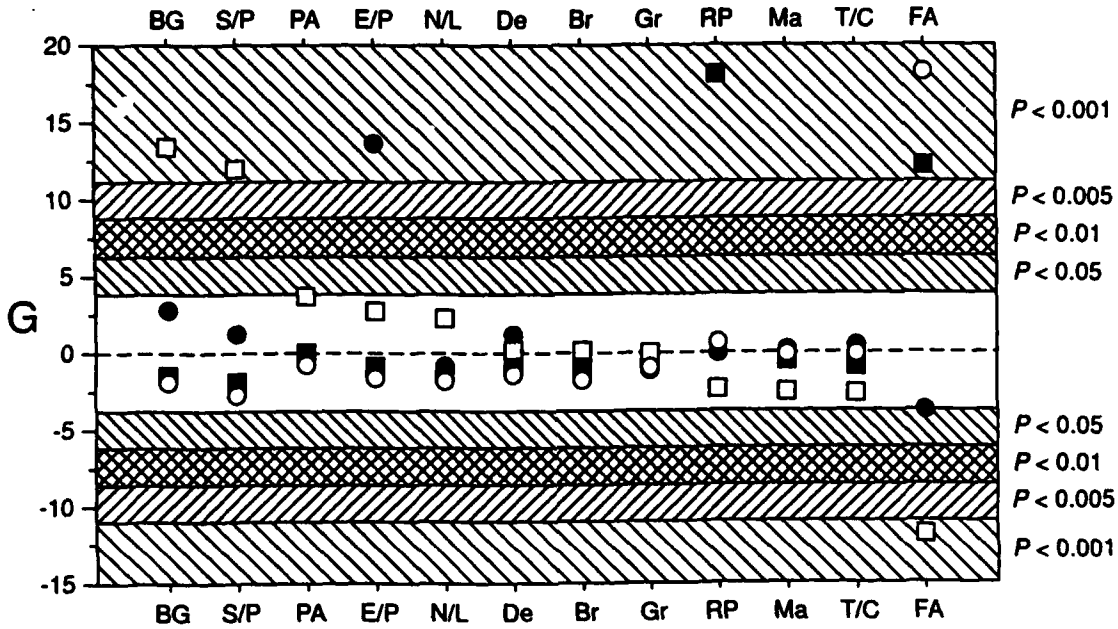

Fig. 4. *G* test of independence between the habitat-types and *Anopheles* larvae present (Belize, April 1991). BG, Cyanobacterial mats; S–P, submersed macrophytes–periphyton; PA, planktonic algae; E–P, *Eleocharis interstincta*–periphyton; N–L, *Nymphaea–Limnanthemum;* De, detritus; Br, broadleaved; Gr, graminoids; RP, rock pools; Ma, mangroves; T–C, *Typha–Cladium;* FA, filamentous algae. Empty square, *A. albimanus;* black square, *A. argyritarsis;* black circle, *A. crucians;* empty circle, *A. pseudopunctipennis.*

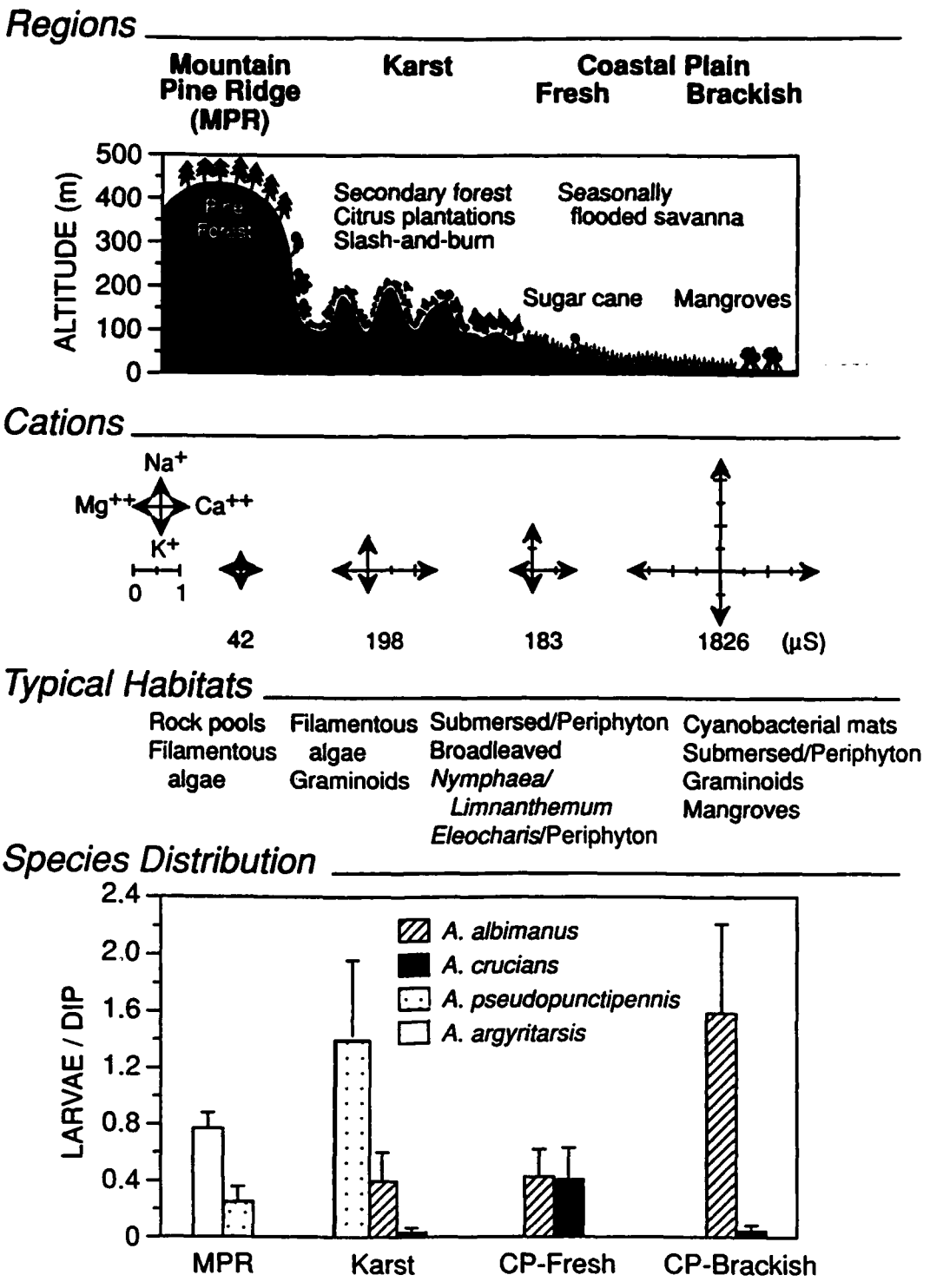

Fig. 5. Mosquito distribution according to physiographic region. Cation concentration is expressed in log mg/liter; numbers below cation diagrams express the specific conductivity. Larval densities for individual species are expressed as the mean number per dip per region; vertical bars indicate the standard error of the mean.

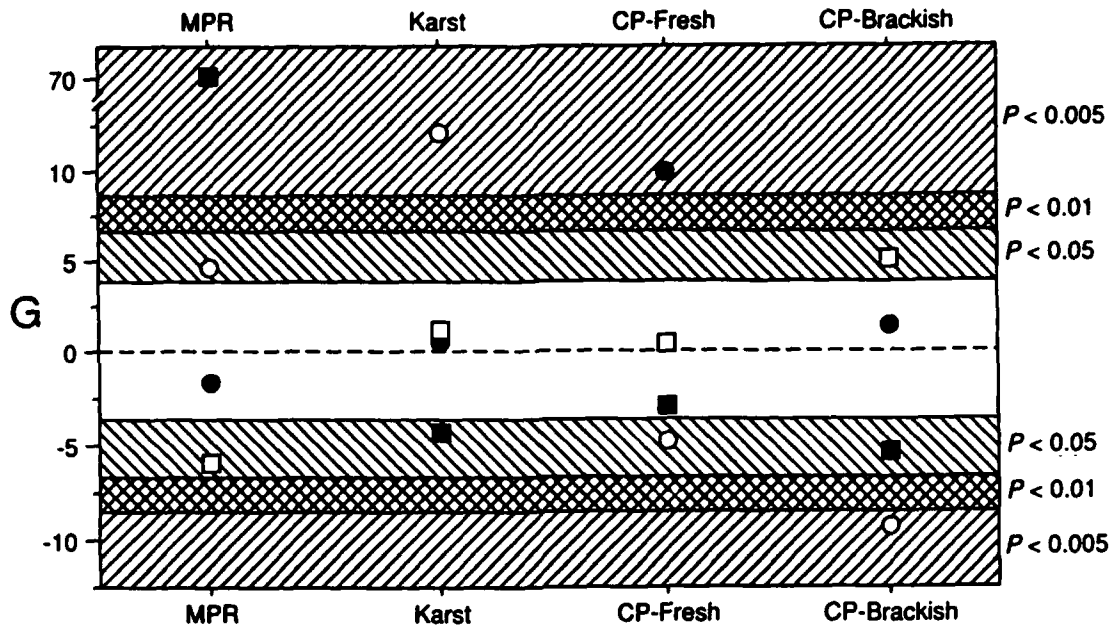

Fig. 6. *G* test of independence between the regions and presence of *Anopheles* larvae (Belize, April 1991). MPR, Mountain Pine Ridge; Karst, Karst and foothill region; CP (fresh), coastal plain, fresh water; CP (brackish), coastal plain, brackish water. Empty square, *A. albimanus;* black square, *A. argyritarsis;* black circle, *A. crucians;* empty circle, *A. pseudopunctipennis.*

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Appendix 1. ***List of plant species related to Anopheles* spp. larval habitats; Belize, wet season, September 1990; dry season, April 1991**

		Season[a]	
		Wet	Dry
Emergent			
Gramineae			
Cynodon dactylon		+++	++
Distichlis spicata		++	–
Gramineae sp.		+++	++
Hymenachne amplexicaulis		++	–
Leptochloa sp.		++	–
Panicum sp.		++	–
Paspalum sp.		+++	+
Paspalum virgatum		++	–
Cyperaceae			
Cladium jamaicense		++	++
Cyperus articulatus		+	+
Cyperus ligularis		+	–
Cyperus odoratus		++	–
Cyperus peruvianum		+	+
Cyperus rotundus		++	–
Eleocharis caribea		++	–
Eleocharis cellulosa		++	–
Eleocharis intersticta		+++	+++
Eleocharis mutata		+	–
Eleocharis sp.		++	+
Fimbristylis spadicea		++	+
Fuirena umbellata		++	+
Rhynchospora barbata		++	–
Rhynchospora cephalotes		+	–
Rhynchospora cyperacea		++	–
Rhynchospora robusta		++	+
Rhynchospora setacea		+	–
Scleria pterata		++	–
Typha domingensis	Typhaceae	++	++

Appendix 1. Continued

	Season[a]	
	Wet	Dry
Broadleaved		
Bacopa monnieri	++	++
Batis maritima	+	–
Echinodorus sp.	++	+
Heteranthera sp.	–	++
Hydrocotyle sp.	+	–
Hymenocalis sp.	++	++
Justicia sp.	++	+
Lippia nodosa	+	+
Ludwigia octovalvis	+++	++
Pontederia sagittata	++	–
Polygonum sp.	++	++
Sagittaria lancifolia	+	–
Spilanthes sp.	++	++
Wedelia sp.	++	–
Rhizophora mangle	++	++
Floating		
Lemna sp.	+	–
Limnanthemum humbolti	++	++
Nymphaea ampla	++	++
Pistia stratiotes	–	+
Algae		
Filamentous	++	+++
Cyanobacterial mats	++	+++
Periphytic	++	+++
Submersed		
Cabomba sp.	–	++
Chara sp.	++	++
Mayaca fluitans	+	–
Naias guadalupensis	++	+
Potamogeton sp.	–	++
Utricularia cornuta	+	–
Utricularia foliosa	++	++
Utricularia resupinata	++	++
Utricularia purpurea	++	++

+++, species occurring frequently; ++, species occurring less frequently; +, species occurring infrequently; –, species not found.

Appendix 2. Detailed Description of Habitat Types

Emergent

Graminoids. Prevalent in the wet season in marshes and seasonally flooded wetlands such as edges of pools and lagoons. Average height above the water surface is 30 cm; often grows to 60 cm; usually not very dense, average cover is 30%. Typical for CP and KARST; fresh waters.

***Eleocharis interstincta*–Periphyton.** A common habitat type in the wet season, present in most depressions in seasonally flooded savanna, usually forming large uniform areas with plants up to 40 cm tall and covering ≈50% of the water. *Utricularia foliosa* as a submersed codominant is quite frequent. This habitat type is less common during the dry season as many habitats become dry. Those dry season habitats with water have senescent *Eleocharis* which is often covered with dense periphyton (Cyanobacteria and Chlorophyta). Typical for CP with fresh or sometimes slightly brackish waters.

Typha–Cladium. Represented by very tall (up to 3 m) and usually very dense (90% cover) emergent macrophytes, mostly *Typha domingensis* or *Cladium jamaicense;* occurring in relatively permanent marshes in both wet and dry seasons and in both fresh and slightly brackish waters.

Broadleaved. Very broad and diverse group of habitats, often containing *Ludwigia octovalvis* as a dominant species. Sometimes low shrubs are present. This, often species-rich, habitat type is generally found on edges of ponds, ditches, and pools and is typical for seasonally flooded areas where aquatic vegetation does not have time to develop. This habitat type is absent in the dry season.

Mangroves. Mostly *Rhizophora mangle* with no other vegetation occurring in salt or brackish waters. This habitat type is present in both wet and dry seasons.

Floating

Nymphaea–Limnanthemum. Floating-leaved macrophytes in more or less permanent fresh waters of ponds and lagoons. Often relatively dense, large, rigid leaves cover the water surface.

Cyanobacterial Mats. Large dense floating mats (scums) consisting of microscopic benthic Cyanobacteria, known also as blue–green algae (e.g., *Phormidium, Lyngbya*). The mats usually develop on the bottom of a water body, then gradually rise to the water surface. Where present, they usually cover large areas. A special microclimate develops in these mats with very pronounced diurnal fluctuations of O_2, pH, and temperature. More frequent in the dry season but also present in the wet season.

Submersed

Submersed Macrophytes–Periphyton. Several species of submersed macrophytes, such as *Mayaca fluitans, Naias guadalupensis, Potamogeton lucens, Chara* spp., often forming dense populations which may break the water surface. This habitat type develops in mostly permanent water bodies, even though some can grow in seasonally flooded roadside ditches and temporary pools. In the dry season, submersed macrophytes are often densely overgrown with periphytic algae.

Filamentous Algae. Predominantly *Spirogyra* species typical of small rock pools in river beds in both MPR and rivers of KARST. Present mainly in the dry season. During the wet season, this habitat type does not have time to develop because river pools are constantly flushed by heavy rains.

Planktonic Algae. Eutrophic water such as cattle ponds; not common and not sampled in the wet season.

Without Vegetation

Rock Pools, No Filamentous Algae. A temporary habitat type present in the dry season in MPR.

Detritus. This habitat type usually develops in small water bodies with fallen leaves and other plant debris.

February 7, 1994

THE SEROEPIDEMIOLOGY OF EPIDEMIC HEPATITIS E IN PAKISTAN AS MEASURED BY A NEW AND SENSITIVE ELISA

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Abstract

In the spring of 1987, 133 people were admitted to a hospital in Sargodha, Pakistan with hepatitis. A new ELISA employing hepatitis E virus (HEV) expressed from a baculovirus vector was used to determine the pattern of IgM and IgG anti-HEV in sera collected from hospitalized patients and their contacts. Of 131 patients hospitalized with non-A, non-B hepatitis, 122 cases (92%) were confirmed as HEV. IgM anti-HEV was detected in 91% of specimens obtained up to 2 weeks before hospitalization and in 100% of sera obtained 5-7 weeks after admission. IgG anti-HEV followed a similar pattern. Peak IgM and IgG anti-HEV titers appeared during weeks 2-4 after hospitalization. Twenty months after hospitalization, IgM anti-HEV was not detected in any of 33 patients, but IgG anti-HEV was found in all. Anti-HEV of the IgG class appeared to be protective in contacts of patients with hepatitis. Thus we have confirmed HEV as the etiology of this outbreak, established the detection of IgM anti-HEV, provided evidence that IgG anti-HEV protects against hepatitis E and demonstrated that IgG anti-HEV persists, but at diminished titer.

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PROGRAM AND ABSTRACTS OF THE JOINT ANNUAL MEETING OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE AND THE AMERICAN SOCIETY OF PARASITOLOGISTS

The Hyatt Regency
Atlanta, Georgia
October 31–November 4, 1993

Supplement to
THE AMERICAN JOURNAL OF
TROPICAL MEDICINE AND HYGIENE

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Enclosure 11

complete recovery after being ventilatated for 7 to 128 hours We are now exploring the use of ancillary pharmacological agents such as 3,4 diaminopyridine and improved antivenoms to prevent or delay life-threatening neurotoxicity.

351 PATTERN OF ANTI-HEV BY ELISA IN AN EPIDEMIC OF HEPATITIS IN PAKISTAN. Bryan JP*, Tsarev SA, Iqbal M, Ticehurst J, Emerson S, Ahmed A, Duncan J, Rafiqui AR, Malik IA, Purcell RH, and Legters LJ. Department of Preventive Medicine, Uniformed Services University of the Health Science, Bethesda, MD; Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD; and Pakistan U.S. Lab for Seroepidemiology, Rawal, Pakistan.

In the spring of 1987, 131 people were admitted to a hospital in Sargodha, Pakistan with non-A, non-B hepatitis. Hepatitis E virus (HEV) was detected previously by immune electron microscopy (IEM) in 10 of these patients. An ELISA employing an antigen expressed in baculovirus from ORF-2 of hepatitis E virus was used to determine the pattern of IgM and IgG anti-HEV in sera collected from hospitalized patients or their contacts on March 20, 1987, and 1 and 20 months later. 122 cases (92%) were confirmed as HEV: IgM anti-HEV was detected in 119 sera obtained before and during hospitalization; two others developed IgG anti-HEV; and in another, HEV was detected in a fecal specimen. Of the remaining 9 hospitalized persons, 7 had normal hepatic enzymes at the time of sera collection (9-30 days before hospitalization in 6), and 2 had IgG but no IgM anti-HEV. IgM anti-HEV was detected in 20/22 specimens obtained up to 2 weeks before hospitalization and in all 18 sera obtained 5-7 weeks after hospitalization. IgG anti-HEV followed a similar pattern. Peak IgM and IgG anti-HEV titers appeared during weeks 2-4 after hospitalization. Twenty months after hospitalization, IgM anti-HEV was not detected in any of 33 patients, but IgG anti-HEV was found in all. IgG anti-HEV in sera at the beginning of the outbreak appeared to be protective; none of the 30 contacts of patients with hepatitis with IgG but no IgM anti-HEV were hospitalized compared with 8 (33%) of 24 who had no IgG or IgM anti-HEV detected ($p = .002$). This ELISA confirms HEV as the etiology of this outbreak, detects IgM anti-HEV as early as 14 days before hospitalization, and detects IgG anti-HEV for at least 20 months after admission.

352 ACUTE FEBRILE ILLNESS IN SOMALIA DURING OPERATION RESTORE HOPE. Magill AJ* and Smoak BL. Department of Immunology, Walter Reed Army Institute of Research, Washington, DC; and Department of Preventive Medicine, Walter Reed Army Institute of Research, Washington, DC.

Acute febrile illness (AFI) was a major cause of hospitalization in U.S. forces deployed to Somalia during Operation Restore Hope. Between 25 February and 01 May 1993, all patients (U.S. forces only) with a temperature > 100.5°F were entered into an AFI study. Ninety-two patients were evaluated with a daily clinical and epidemiological history, physical examination, complete blood count, and malaria smears. Serum was obtained on admission (early acute), discharge (late acute), and at least one month after admission (convalescent). All cases were arbitrarily divided into 4 categories: dengue or dengue like illness in 45 of 92 (49%), gastroenteritis in 18 of 92 (20%), malaria in 6 of 92 (7%) and miscellaneous in 23 of 92 (25%). Of the 45 suspected arboviral cases, 10 (22%) were classified as dengue based on a strongly positive IgM antibody-capture assay. The cases were sporadic and occurred throughout Somalia. *Shigella* was isolated in 3 cases of gastroenteritis. The malaria cases (3 [*P. falciparum*], 1[*P. vivax*], 1[*P. malariae*] and 1 undetermined) were characterized by low parasitemias and mild clinical presentations. The miscellaneous category included pharyngitis, sinusitis, pneumonia, abdominal pain, mononucleosis, hidradenitis, cellulitis, heat exhaustion and drug hypersensitivity. There were no deaths and no complications due to malaria or any other febrile illness during the 65 day study period. Clinically suspected arboviral infections were the leading cause of febrile admissions during this study period.

INTERNATIONAL SYMPOSIUM ON VIRAL HEPATITIS AND LIVER DISEASE

(The 8th Triennial Congress)

SCIENTIFIC PROGRAM AND ABSTRACT VOLUME

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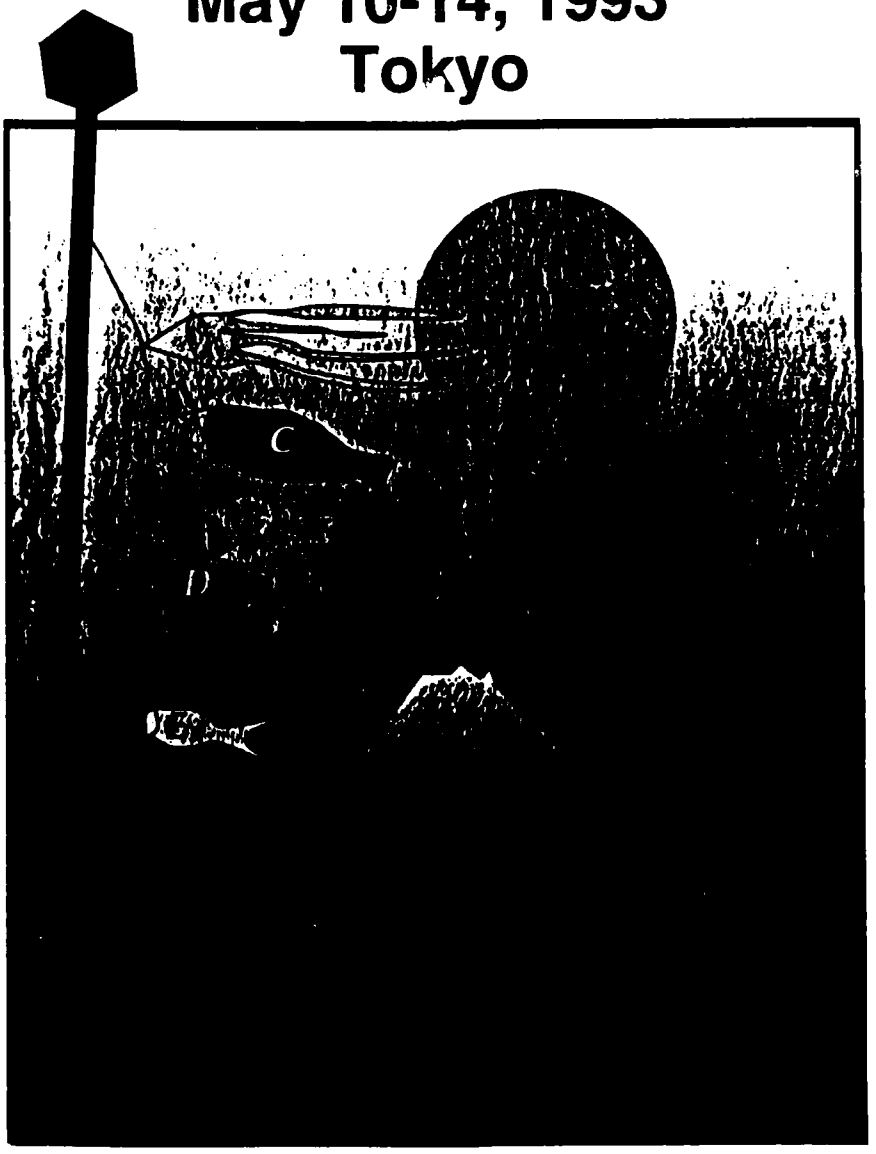

VIRAL HEPATITIS RESEARCH FOUNDATION OF JAPAN (VHFJ)

Enclosure 12

ABSTRACT[§]

PATTERNS OF HEPATITIS E VIRUS (HEV) EXCRETION FROM SELECTED PATIENTS IN AN OUTBREAK. HY Zhang, JD Caudill, TJ Popkin, JF Duncan, I Hussain, A Ahmed, M Iqbal, IA Malik, LJ Legters, J Ticehurst. Walter Reed Army Inst Research, Washington DC, National Insts Health & Uniformed Services Univ Health Sci, Bethesda MD, USA, Army Medical College, Rawalpindi, Pakistan.

Objectives/methods: An earlier study determined, by immune electron microscopy (IEM) of single specimens, that HEV was predominantly excreted during the 1st week of jaundice [J Med Virol 36:84-92, 1992]. During a 1988 outbreak, multiple feces were collected from 19 patients. To increase the likelihood of detecting HEV and to determine excretion patterns, early acute-phase collections were assayed by affinity-capture/polymerase chain reaction (AC/PCR). Results: HEV was detected 3-16 days into icterus, but only 29% of specimens were AC/PCR-positive (Table). HEV excretion was intermittent from 2 patients. HEV was detected in 4 of 10 specimens diluted 10^{-4}. 1 of 2 samples had an AC/PCR titer of 10^{-6}. Discussion: HEV excretion was detected from 5 of 11 patients and during the 3rd week of illness. However, HEV concentrations were usually very low, at or below IEM-detectable levels. Such data were consistent with earlier IEM and PCR results. Case #13 was anti-HEV IgM-negative [Ticehurst et al, submitted for this symposium]; thus, AC/PCR could be used for diagnostic support when suspected cases are anti-HEV IgM-negative.

Table. AC/PCR*: detection of HEV in feces from Abbottabad, Pakistan

	Days after onset of scleral icterus															
Case #	2	3	4	5	6	7	8	9	10	11	12	13	16	19	22	25
1				-	-											
4				-												
7					-											
9					+	-	-									
11					-											
13					+	+										
63		-														
64		-	+	-	-	-				-						
65			-		-	-	-				-					
67	-	+	-		-							-	+			
68	-	+	+	+			-		+			-	+	-	-	-

Certain specimens from this group were assayed by IEM: HEV particles were not detected
* TA Miele et al, abstract submitted for this symposium

[§] Errors have been corrected in the table (Cases 7, 9, 11, 13, 63)

ABSTRACT*

ANTIBODY TO HEPATITIS E VIRUS (ANTI-HEV) IN SERIAL SERA FROM AN OUTBREAK: DETECTION BY ENZYME IMMUNOASSAY (EIA).

J Ticehurst, PO Yarbough, JP Bryan, E Garza, JD Caudill, AR Rafiqui, A Ahmed, M Iqbal, IA Malik, JF Duncan, LJ Legters. Walter Reed Army Inst Res, Washington DC, Genelabs Diagnostics, San Antonio TX, Uniformed Services Univ Health Sciences, Bethesda MD, USA, Army Medical College, Rawalpindi, Pakistan.

Objectives/methods: A hepatitis outbreak during August 1988 resulted from fecal contamination of the water supply for a housing unit. To determine patterns of antibody reactivity, EIA (Table) was used to test 27% of the sampled individuals. Results: Anti-HEV IgM was detected in 43% of Cases during Aug; IgG, in 97%. By Sept, "seroconversions" occurred: IgM developed in 5 Cases; IgM and IgG in 1 Normal; IgG in 1 Case and 2 Contacts. Conversely, IgM was no longer detected in 2 Cases; IgG was not detected in 1 Normal who had IgG in Aug and Dec. By Dec, IgM was no longer detected in 100% of Cases; 2 Cases (11%) and 1 Contact no longer had IgG. Case #7 had IgG and IgM in Aug but neither in Dec; by immune electron microscopy (IEM), both sera had high levels of anti-HEV. Discussion: Anti-HEV IgM was detected in 61% of Cases within 1 month of jaundice and in 0% by 4 months. Thus, EIA readily identified HEV as the probable cause of the outbreak; it was also shown [H-Y Zhang et al, submitted for this symposium] that Cases, including an IgG+/IgM- patient, excreted HEV. We did not determine if other IgG+/IgM-results indicated current or past infection, or nonspecificity. In general, however, IgG was most frequently detected in Cases. Development of anti-HEV among non-cases suggested that anicteric infections occurred. It appeared that anti-HEV IgG sometimes waned, consistent with earlier results. However, the significance of the EIA-IEM discrepancy for Case #7 is not known.

Table. EIA[a]: detection of anti-HEV in sera from Abbottabad, Pakistan[b]

	Cases		Contacts		Normals	
	IgM	IgG	IgM	IgG	IgM	IgG
Aug	11/26[c]	29/30	0/10	2/10	0/16	3/16
Sept	8/18	17/17	0/7	3/7	1/10	2/10
Dec	0/19	17/19	0/3	2/3	0/1	1/1

[a] R Goldsmith et al, Lancet 339:328-331, 1992

[b] Collected in Aug, Sept, and Dec 1988 (most individuals contributed 2 or 3 sera) from Cases (jaundiced patients), Contacts (ill but not icteric), and Normal persons

[c] No. positive / no. assayed

* An error in the table (fraction for Cases/IgM/Aug) has been corrected

Prevalence Of Sand Fly Fever, West Nile, Crimean-Congo Hemorrhagic Fever and Leptospirosis Antibodies In Pakistani Men

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ABSTRACT

To determine the prevalence of antibody to viral diseases known or suspected to be present in Pakistan, we studied 570 sera from three groups of adults; two of the groups were involved in outbreaks of enterically transmitted non-A, non-B hepatitis, and the third included men admitted to a hospital for evaluation of febrile illnesses. IgG anti-leptospiral antibody was found in 1-6% of the subjects, with the highest rate in enlisted military personnel hospitalized for febrile illness. Only one man in the group with febrile illness had significantly elevated IgM anti-leptospiral antibody titers. However, in a group of recruits experiencing suspected non-A, non-B hepatitis, 19 (11%) of 173 had a four fold rise in IgM antibody to leptospirosis. Antibody to sand fly fever viruses was found in 43-76%. The lowest prevalence, 26%, was observed in a subset of subjects ≤ 19 years old attending a service academy. Antibody to West Nile virus, which causes a febrile illness with rash and encephalitis, was present in 37-46% of subjects. Antibody reactive with Japanese B encephalitis virus was present in 32-35%, but in almost all cases, it appeared to be cross-reactive with West Nile virus. All 212 specimens tested for Crimean-Congo hemorrhagic fever virus were negative. This study indicates that diseases known to be prevalent in other areas of Southwest Asia and the Middle East are also prevalent in Northern Pakistan and may impact on those traveling or working in the area.